# ANTI-BACTERIAL PROPERTIES OF ETHANOLIC *MORINGA OLEIFERA* LEAF EXTRACT AND PROTEOMIC ANALYSIS OF ITS EFFECTS ON *ESCHERICHIA COLI*

A Thesis
by
BRANDON EDWARD SMITH

Submitted to the Graduate School
at Appalachian State University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

August 2016
Department of Biology

ANTI-BACTERIAL PROPERTIES OF ETHANOLIC *MORINGA OLEIFERA* LEAF EXTRACT AND PROTEOMIC ANALYSIS OF ITS EFFECTS ON *ESCHERICHIA COLI*

A Thesis
by
BRANDON EDWARD SMITH
August 2016

APPROVED BY:

Chishimba Nathan Mowa, Ph.D.
Chairperson, Thesis Committee

Ece Karatan, Ph.D.
Member, Thesis Committee

Sue Bauldry, Ph.D.
Member, Thesis Committee

Zack Murrell, Ph.D.
Chairperson, Department of Biology

Max Poole, Ph.D.
Dean, Cratis D Williams School of Graduate Studies

**Abstract**

ANTI-BACTERIAL PROPERTIES OF ETHANOLIC *MORINGA OLEIFERA* LEAF EXTRACT AND PROTEOMIC ANALYSIS OF ITS EFFECTS ON *ESCHERICHIA COLI*

Brandon Edward Smith
B.S., Appalachian State University
M.S., Appalachian State University

Chairperson: Chishimba Nathan Mowa, Ph.D.

Every year 15 million babies are born prematurely worldwide and a million of these infants die. We know that bacterial infections are associated with and are the primary cause of preterm labor. Unfortunately, current antibiotic-based therapies are either unsafe or are becoming less effective due to the development of bacterial resistance to these therapies. Some of the key microbes associated with preterm labor include *Gardnerella vaginalis, Lactobacillus spp., Atopobium vaginae, Mycoplasma hominis, Ureaplasma urealyticum* and *Escherichia coli (E. coli).* Here, we investigated the effectiveness of different whole leaf *Moringa oleifera* (*M. oleifera*) extracts on *E. coli,* comparing the most potent leaf extract to common antibiotics. We sought to determine the genome-wide protein expression patterns taking place in the bacterium when incubated with whole leaf *M. oleifera* extract by using quantitative proteomics. From these studies, we demonstrate 1) ethanolic whole leaf *M. oleifera* extract causes greatest inhibition of *E.coli* and is comparable to inhibition observed by common antibiotics, 2) different phases of bacterial growth are prolonged or inhibited by extract treatment, and 3) quantitative proteomics revealed vast changes in proteins associated with distinct biological processes such as stress response, metabolism and energy maintenance.

**Acknowledgments**

I would like to acknowledge the following sources of funding for this study: Appalachian State University Office of Student Research, Cratis D. Williams Graduate School, Appalachian State Summer Ventures in Science and Mathematics Camp, Appalachian State Future Doctor's Camp. I wish to acknowledge Dr. Chishimba Nathan Mowa for his patience, guidance, and wisdom during the past two years. I wish to acknowledge and thank my colleagues in our research lab, Takako Ohashi, Casey Colicchio, Alicia Brunson, Morgan Fitch, Savannah Ray, Cole Sloboda, Jacob Gordon, and Tanner Orders. I would also like to send a special thanks to Lori Tyler, Director of Summer Ventures and Future Doctors camps and the students that assisted me during the summers including Vernon Espinoza, Sri Nair, Vivek Ravi, and Jetha Tallapaneni. I wish to acknowledge my thesis committee members, Dr. Ece Karatan and Dr. Sue Bauldry for their assistance during my graduate career. I wish to thank Dr. Guichuan Hou and Dr. David Kirchner for their assistance in microscopy and proteomics. I wish to thank my friends and family, Mom, Dad, and Justin for their love and support.

## Dedication

I wish to dedicate this work to my family for making me the person I am today, and for supporting me through all my endeavors regardless of knowing the purpose of many of them. To my father, Mickey Smith, for earning an honest wage and for teaching me the importance of respect, hard work, and dedication is necessary to aspire to ones goals. To my mom, Debbie Smith, for being understanding and your guidance in all aspects of life; you are one of a kind in both of those aspects. To my younger brother, Justin Smith, for giving me someone to look up to, always pushing me to be better, and being such a great friend.

I also wish to dedicate this work to my friends from home and those that I have made during my studies at App. Through everything, successes, and failures you guys have always been there making this journey memorable in so many ways. I love all of you for that and will never forget the times we've had and look forward to those we will surely enjoy together.

## Table of Contents

## List of Tables

## List of Figures

## Introduction

*Purpose of Study:*

Every year 15 million babies are born prematurely worldwide and a million of these infants die (Beck et. al., 2009). We know that bacterial infections are associated with and are the primary cause of preterm labor (Agrawal & Hirsch, 2012). Unfortunately, current antibiotic-based therapies are either unsafe or are becoming less effective due to the development of bacterial resistance to these therapies. Some of the key microbes associated with preterm labor include *Escherichia coli (E. coli), Gardnerella vaginalis, Lactobacillus spp., Atopobium vaginae, Mycoplasma hominis,* and *Ureaplasma urealyticum* (Beck et. al., 2009; Klein & Gibbs, 2004). Because of these reasons, as well as the fact that more than 80% of the world's population depends on medicinal plants for their primary health, the purpose of the present study is to begin to test the effectiveness of widely accessible natural products that have been safely used for thousands of years. *Moringa oleifera* (*M. oleifera*) fits this description and its leaf extract was tested on inhibiting bacterial growth. Specifically, we used *E. coli* as a model microbe and attempted to delineate the underlying mechanism of *M. oleifera's* anti-*E. coli* activities using basic bacteriological techniques, as well as proteomics, which in future studies will be verified by Western blot and real time PCR analysis, respectively. The primary hypothesis of the present study was that *M. oleifera* can be used to inhibit the growth of one of the key bacteria associated with preterm labor, *E. coli*. If successful, insights gained from the current *in vitro* study will be used to develop *in vivo*-based studies using mice models, with hopes of extending the studies to humans in the future.

*Overview:*

Systemic and local bacterial infections have been linked to and believed to be the major cause of premature labor (Beck et al., 2009; Klein & Gibbs, 2004). Premature labor is a common phenomenon and represents a costly health care condition around the world (Blencowe et al., 2012). Every minute more than 100 infants die from premature births, while over 1,400 babies are born prematurely and many will live with and suffer from life-long side effects (Beck et al., 2009; Reddy et al., 2012). For now, the exact mechanisms that lead to premature fetal delivery are not completely understood. However, we do know that premature delivery is largely caused by microbial infection that subsequently triggers an inflammatory response (El-Bastawissi et al., 2000; Romero, Avila, Santhanam, & Sehgal, 1990; Romero et al., 2006).

Bacteria, such as *Escherichia coli, Gardnerella vaginalis, Lactobacillus spp., Atopobium vaginae, Mycoplasma hominis,* and *Ureaplasma urealyticum,* have the ability to colonize the placenta during fetal development through many different routes, including the urinary and circulatory systems, and, most commonly, via ascension through the vagina to fetal membranes and amniotic cavity (Beck et al., 2009; Cram, Zapata, Toy, & Baker, 2002; Klein & Gibbs, 2004; Romero et al., 1990). Upon penetration of the bacteria into the epithelial cells of the cervix and vagina, the bacterium binds to toll-like receptors (TLRs) and subsequently trigger the recruitment of neutrophils and monocytes, eventually leading to an increase in cytokine production (Adib-Conquy, Scott-Algara, Cavaillon, & Souza-Fonseca-Guimaraes, 2014; Klein & Gibbs, 2004). Toll-like receptors are membrane-bound proteins that recognize foreign invaders, including bacteria, and are believed to play a crucial role in infection-mediated preterm birth (Challis et al., 2009).

Urinary Tract Infections (UTIs) are very common during pregnancy and *E. coli* is the key contributor of UTIs (Ovalle & Levancini, 2001). *E. coli* is also one of the most common pathogenic bacteria associated with preterm labor and isolated from pregnant women (Ovalle

& Levancini, 2001). Antibiotics, such as tetracycline and ciprofloxacin, are commonly used to treat *E. coli*-induced UTIs (Blair, Webber, Baylay, Ogholu, & Piddock, 2015). However, the problems associated with use of antibiotics are resistance and safety, particularly during delicate biological events, such as pregnancy (Blair et al., 2015). Other than posing health risks and resistance, use of antibiotics, especially in poor countries or communities, is also more costly or may not be available (Blair et al., 2015). Since 80% of the world's population rely on medicinal plants, it is essential to test the efficacy of medicinal plants in treating infection-induced preterm labor (Fahey, 2005).

Thus, new therapies that are both safe to the fetus and mother and effective against preventing bacterial infections are needed to combat preterm labor. Historically, plant extracts have been used for a long time as medical ointments for treating many illnesses, and continue to be used to this day, especially in developing countries, where modern medicine is either too costly or perhaps unavailable. Various conditions treated with plant extracts include infection, inflammation and pain (Fahey, 2005; Kasolo, Bimenya, Ojok, Ochieng, & Ogwal-Okeng, 2010). *M. oleifera* has been used safely as a medicinal plant world-wide for thousands of years (Mbikay, 2012). It has been reported to help reduce a plethora of illnesses, including inflammation, high cholesterol, UTI infection and depression, while boosting the immune system and energy (Charoensin, 2014; Konda, Madhavi, Ruckmani, & Venkataramana, 2013; Mbikay, 2012).

*M. oleifera* has lately received increased attention for its medicinal and nutritional properties and several of its bioactive phytochemicals have been identified, including catechol tannins, gallic tannins, steroids and triterpenoids, flavonoids, saponins, anthraquinones, alkaloids reducing sugars and many more (Fahey, 2005; Kasolo et al., 2010; Muhammad, Pauzi, Arulselvan, Abas, & Fakurazi, 2013). Various *M. oleifera* leaf extracts have also been shown to contain many bioactive compounds that exert anti-proliferative properties against bacteria such as *E. coli, Staphylococcus aureus, Vibrio cholerae, and Salmonella enteritidis*. These bioactive

compounds (with anti-proliferative properties) include benzyl isothiocyanate, 4-benzyl isothiocyanate, niazimicin, pterygospermin, and 4-benzyl glucosinolate (Bukar, Uba, & Oyeyi, 2010; Fahey, 2005; Mahbub, Hoq, Ahmed, & Sarker, 2011; Oluduro, 2012), and appear to be unique to the *Moringa* genus, making *M. oleifera* a potential treatment of choice for infection-induced preterm labor (Fahey, 2005). Indeed, bacteria, such as *E. coli, S. aureus, Pseudomonas aeruginosa, and Proteus mirabilis* that are associated with preterm labor, have been shown to be hindered by *M. oleifera* leaf extract's anti-bacterial properties in non-reproductive tissues (Abraham & Okon, 2014; Bukar et al., 2010; Fahey, 2005; Mahbub et al., 2011; Oluduro, 2012; Rahman et al., 2009; Tiloke, Phulukdaree, & Chuturgoon, 2013). Based on these studies, it is reasonable to speculate that *M. oleifera* could potentially be used to prevent bacterial infection-induced preterm labor. It is also rational to speculate that *M. oleifera* could potentially be regulating bacterial replication by preventing key biological processes from occurring.

In the present study, we examined the antibacterial properties of *M. oleifera* against *E. coli*, which is commonly associated with urinary tract infection and causes around 20% of premature births (McGuire, 2004). We also screened the expression of key proliferation-associated proteins using proteomics. A non-pathogenic strain of *E. coli*, DH5α (lacking type 1 fimbriae), will be used as a model organism for the pathogenic *E. coli* which has fimbriae that allow it to adhere to the human uroepithelium.

*Objectives:*

Here, we attempted to characterize the anti-*E. coli* activities of *M. Oleifera* by: 1) testing the effectiveness of five different whole leaf extracts of *M. oleifera* to prevent bacterial growth in an *in vitro* model and compared the extracts to the commonly prescribed antibiotics for *E. coli*, primarily Streptomycin and Tetracycline and 2) delineated the likely underlying molecular

mechanisms of *M. oleifera's* anti-*E. coli* activities by identifying the expression of the signature factors that are involved in key biological processes using proteomics analysis.

## Materials and Methods

*Microbe Used:*

A non-pathogenic strain of *E. coli*, D51α, generously provided by Dr. Ece Karatan (Department of Biology, Appalachian State University, Boone, NC), was used in this project due to its ease in growing and safety. In all the experiments conducted in the present study disposable sterile petri dishes (VWR, Radnor, Pennsylvania, USA) with LB agar (AMERCO, Ohio, USA) were used. The petri dishes were divided into 4 quadrants, streaked and labeled (Figure 1). Thereafter, the petri dishes were placed in a 37 °C incubator for about 24 hours to allow sufficient growth of bacterium. Single bacterial colonies obtained from these experiments were then placed in liquid culture to be used in later procedures.

*Preparation of Agar Plates:*

To make agar plates, 22 grams of premix LB Agar Powder (Ameresco, Solon, Ohio, USA) was weighed and added to a flask containing 250 mL of distilled-water ($dH_20$), and thereafter thoroughly mixed and autoclaved (Steris Ameresco, Ohio, USA) at 121°C for 20 minutes, before cooling for approximately 20 minutes. About 12.5 mL of the cooled agar solution was then added to each of the 20 sterile petri dishes (VWR, Radnor, Pennsylvania, USA) that were placed on a sterilized table. The plates were allowed to cool for another 25 minutes and the dishes were then placed in a refrigerator set at 4 °C for storage.

*Preparation of Glycerol Cultures:*

Liquid bacterial cultures were used throughout this experiment in order to limit contamination of plate cultures due to repeated exposure to the environment. A single colony of bacteria was scrapped from the petri dish and carefully inoculated into 2 mL of LB (Amresco, Solon, Ohio) broth using aseptic techniques. The inoculated broth culture was then placed in a 37°C incubator for 24 hours and thereafter stored at -80°C. Bacteria cultures were then counted for colony-forming units by doing serial dilutions and plating. A consistent concentration of 1500 CFU/µL was maintained for stock bacteria cultures. The cultured bacteria was then pelleted and re-suspended in 2 mL of 15% glycerol solution for storage. Each stock solution was used for three experiments by adding bacteria to LB (Amresco, Solon, Ohio) broth and then discarded to limit contamination.

*Preparation of Stock M. oleifera Extracts:*

The whole leaf extracts of *Moringa* were generously provided by our collaborators, Drs. Joshua Idassi and Jahangir Emrani of North Carolina A &T University (NC A & T), Greensboro, NC, where the plant was grown, harvested and processed. Briefly, after harvest, the leaves were carefully dried, blended into powder and then dissolved in 5 different solvents (ethanol, ethanol/water (80/20), methanol, butanol and water) for extraction. The solvents were then evaporated through rotary evaporation. The final extracts were then suspended in sterile 0.1 M PBS (Table 1).

*Disk Diffusion Assay:*

Aseptic techniques were used throughout the present study in all procedures. To prepare the diffusion assay, 200 µL of stock *E. coli*/LB (1500 CFU/µL) broth was spread on an LB agar petri dish. The dishes were then allowed to dry for 10 minutes. Sterile discs (3) were

then evenly placed throughout the petri dish. The appropriate amount (amount to be used depended on experiment) of *M. oleifera* extract solution was then pipetted onto each of the disks and, the dishes were incubated overnight at 37 °C. After incubation, the diameter of inhibition zones were then measured in millimeters (mm) by using a ruler, with the smallest unit estimated at ½ mm (Figure 2).

*Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentrations:*

In order to determine the minimum inhibitory concentration (MIC), different concentrations of *M. oleifera* plant extract were added to LB agar plates with *E. coli*, as explained earlier under the diffusion assay protocol above. Plates were then incubated for 24h at 37°C and examined for microbial growth. Plain solvents, lacking the plant extracts (ethanol, methanol, butanol, water), as well as 0.1M PBS (final solvent after evaporation of initial solvents), were used as controls. The MIC was defined as the lowest concentration of the extract at which an inhibitory zone was first detected.

In order to determine the minimum bactericidal concentration (MBC), different concentrations of *M. oleifera* plant extract were added to tubes containing 2 mL of LB medium plus 20 µL cell culture (1500 CFU/µL). After incubation (37°C for 24h), an aliquot (10 µL) of each sample that did not show visible growth were inoculated onto plates containing LB agar. Plates were incubated for 24h at 37°C and then examined for microbial growth. Again, plain solvents (ethanol, methanol, butanol and water), as well as 0.1M PBS were used as controls. The MBC was defined as the lowest concentration of the extract at which no microbial growth was detected.

*Lethal Dose 50% ($LD_{50}$):*

$LD_{50}$ studies were then conducted in order to determine the extract with the most potent anti-bacterial effects, as well as compare the potency of the 100% ethanol *Moringa oleifera* extract versus select antibiotics (Tetracycline, ampicillin and streptomycin). The cell concentration of *E. coli* added to the broth culture was 20 µL (1500 CFU/µL). All the experimental groups were incubated at 37°C and shaken at 200 rpm in an incubator for 24h. $LD_{50}$ was determined as the absorbance ratio of the control group (bacteria with no treatment) versus experimental groups at $OD_{600}$ using a spectrophotometer. The $LD_{50}$ was the concentration at which the experimental groups' absorbance was half that of the control group's absorbance. Each experiment was carried out in triplicate.

*Antibacterial Kinetic Plots:*

Following the determination of MIC and MBC, we then examined the antibacterial kinetics of *M. oleifera* against *E. coli*. The experimental set up, as well as concentrations of *M. oleifera* were as follow: **a**) Negative control (vehicle only); **b**) Treatment groups: Dose-dependent treatments of *M. oleifera*: **i**) 0.75 µg/mL, **ii**) 1.5 µg/mL and **iii**) 3 µg/mL, respectively; **c**) Positive control groups: Streptomycin, tetracycline, and ampicillin were used as positive (antibiotic) controls using their respective $LD_{50}$ concentrations. The cell concentration of *E. coli* added to the broth culture was 10 µL/mL (1500 CFU/µL). All the experimental groups (controls and treatment groups) were then incubated at 37°C and shaken at 200 rpm in an incubator with a shaker. At the following time points: 0, 1h, 2h, 4h, 8h, 16h, 24h, 48h and 72h, the absorbance in each 2 mL culture was measured in order to depict a growth pattern of the incubated *E. coli*. The antibacterial kinetic patterns were made by measuring optical density at 600 nm ($OD_{600}$) determined using a spectrophotometer. Each experiment was performed in triplicates.

*Proteomics:*

Following the basic microbial and morphological studies, we then sought to investigate the underlying mechanism of *Moringa's* effects on *E. coli* using proteomics.

Sample preparation and treatments: Briefly, samples were prepared by adding 2 mL of LB broth to six tubes (Triplicates studies for both negative control and treated groups). Twenty µL of *E. coli* was then added at 1500 CFU/µL and placed in an incubator to allow growth for a total of 24 h at 37˚C and rotation of 200 rpm. Control samples (bacteria alone) were left in the incubator for the entire 24 h without any additional treatment. For the treatment group 2.5 µg/mL of ethanolic *M. oleifera* whole leaf extract was added to each sample at 16 h of incubation and the samples were immediately placed back in the incubator for the remaining 8 hrs. Samples were then recovered from the incubator and placed in -80˚C at the end of the 24 h incubation. Protein was then extracted from the samples (as described below) and then sent to the David Murdock Research Institute at Kannapolis, NC, for proteomics analysis. The remaining portion of the proteomic methods were performed at the David Murdock Research Institute by David Kirchner.

Protein extraction and sample concentration: Total protein for proteomics was extracted from 6 samples, 3 control and 3 treatment, of *E. coli* cells using the protein inhibitor cocktail and cell lysis buffer from Sigma Aldrich (St Louis) and the supernatant was immediately stored at -80°C. The core facility Laboratory of the David Murdock Research Institute (Kannapolis, NC) performed the proteomics analysis and selected proteins from the proteomics data were then analyzed.

Protein samples were concentrated using 3 KDa Molecular Weight Cut-Off (MWCO) spin filters (Amicon) following the manufacturers protocol for concentrating protein samples. The entire volume of each sample (400-500 µL each) was loaded into clean, separate spin filter devices and centrifuged at 14,000g for approximately 30 minutes until volumes were reduced

to approximately 20 µL each. The filters were then inverted and placed into clean collection tubes and centrifuged for 2 minutes at 1,000 g to collect the protein concentrate. After the samples were concentrated, they were dried down in a vacuufuge for 40 minutes at 30°C. The protein samples were reconstituted in 100 µL of LC/MS grade $H_2O$ and briefly sonicated, vortexed and then centrifuged. The samples were then transferred into clean, labeled micro-tubes and BCA assays were performed.

Micro BCA assay: The protein concentration of the 6 samples was determined using a microBCA assay (Thermo) following the manufacturers protocol.

Reduction, Alkylation and Trypsin Digestion: The results from the first trial microBCA assay were used to measure out 10 µg of each protein sample for enzyme digestion and MS analysis. Then LC/MS grade water was added to bring each sample to a concentration of 2µg/µl. Two (2) µL of 10 mM Dithiothreitol (DTT) was added to each sample tube and the tubes were incubated at 80°C for 30 min. Then 2µL of 200 mM Iodoacetamide (IAA) was added and tubes were incubated at room temperature in the dark for 20 min. One (1) µL of a 200 ng/µL Trypsin solution in 50 mM Ammonium Bicarbonate (AmBiC) was added and the tubes were incubated for 5 h at 37°C then overnight at 4°C. The reactions were quenched by adding 1 µL of 10% acetic acid to the reaction tubes. The tubes were then briefly centrifuged to place any condensate from incubation at the bottom of the tubes. The samples were vacuufuged for 30 min at 30°C. Each sample was then reconstituted in 25 µL liquid chromatography loading buffer (2% Acetonitrile, 0.1% formic acid), and briefly sonicated to solubilize the peptides, and the whole 25 µL of sample was transferred into clean, labeled LC/MS vials for data collection and analysis. The generation of a pooled quality control (pQC) sample was accomplished by transferring 3.0 µL of each peptide sample in a clean, labeled LC/MS vial.

Sample preparation for the second trial was similar to the first trial reaction setup, but using the second Trial microBCA results that were obtained after sample concentration using 3

KDa MWCO spin filter and resuspension. Additionally, the reactions in this trial were normalized to equal 20 µg of protein per sample, with the amount of water needed calculated by subtracting the needed protein sample amount from 10.0 µL. As before, 3µL of 10 mM DTT was added to each sample and the tubes were incubated at 80°C for 30 min. Three (3) µL of 200 mM IAA was added and tubes were incubated at room temperature in the dark for 20 min. Then 2 µL of 200 ng/µL Trypsin solution in 50 mM AmBiC was added and the tubes were incubated for 5 hours at 37°C then overnight at 4°C. The reactions were quenched by adding 1 µL of 10% acetic acid to the reaction tubes. The tubes were then briefly centrifuged to place any condensate from incubation at the bottom of the tubes. The samples were vacuufuged for 30 min at 30° C and dried down. Each sample was then reconstituted in 20 µL liquid chromatography loading buffer (2% ACN, .1% FA) and briefly sonicated to solubilize the peptides. All 20 µL of sample was transferred into clean, labeled LC/MS vials for data collection and analysis. The generation of a pooled QC (pQC) sample was accomplished by transferring 3.0 µL of each peptide sample in a clean, labeled LC/MS vial.

Capillary Chromatography and LC-MS/MS Analysis: Three µL of each sample was injected for analysis on an LTQ-Orbitrap XL MS. First, on-line reversed phase C18 sample trapping, cleanup and focusing was employed for the first 10 min of each analysis. Then, a 33 min elution gradient was used for analytical C18 separation of the tryptic peptides. Fifty femtomoles of standard Yeast Enolase tryptic digest was injected as a QC sample to monitor instrument stability at the beginning and the end of data collection.

The MS method employed was Full scan profile MS at 60,000 resolution 350 - 1800 m/z followed by top 3 in abundance selection for centroided tandem Collision Induced Dissociation (CID) MS and a decision tree based Electron Transfer Dissociation (ETD) activation fragmentation option.

Database Searching and PEAKS Analysis: Data analysis was done by importing the RAW data files into PEAKS (BSI Software). Parameters for the PEAKS analysis can be found in Table 2 (Table 2).

*Statistical Analysis:*

Data were analyzed using Student's *t* test and ANOVA (single factor). *P*-values ≤ 0.05 were considered to be statistically significant.

Proteomics: The aligned mass features were annotated with the database search results using the results from the system Peptide Tellers and a predicted error rate of 1%. MS data were summarized to the feature level, normalized, and an error-weighted ANOVA test was performed to compare the expression results between sample groups. Candidate differentially expressed markers were determined based on a $p<0.01$. Biological processes each protein was involved in were summarized by protein based on the results of the database search and previous literature on proteomics.

## Results

*Only Ethanol and Hydro-ethanol Leaf Extracts of M. oleifera Inhibit Bacterial Growth Based on Diffusion Assay:*

All five *M. oleifera* extracts were tested at the same concentration to determine the extract with the most potent antibacterial activities based on the diameter of the inhibition zones (Figure 3). According to data from the diffusion assay experiments, only two *M. oleifera* extracts out of the 5 different solvents exhibited antibacterial properties by preventing *E. coli* growth based on the diameter of the inhibition zones, with the most significant inhibition (greatest diameter) displayed by both ethanol-based solvents (Figure 3). Of the two ethanol-based extracts, the pure ethanol extract exhibited a greater inhibition than the hydro-ethanol extract (80% ethanol) by approximately 2-fold magnitude in inhibition. The rest of the extracts, including butanol, methanol and water extracts displayed an inhibitory zone with a diameter of 6 mm (Figure 3), which was indicative of zero inhibition of *E. coli* growth (bacterial growth all the way up to the disc (Figure 3)).

*Ethanol-based Leaf M. oleifera Extracts Inhibit E. coli Growth in a Dose-dependent Manner:*

Since the ethanol-based *M. oleifera* extract showed the highest antibacterial effects, we next conducted experiments aimed at determining the minimal concentration of *M. oleifera* required to inhibit the growth of *E. coli* and whether it was dose-dependent (Figure 4). The minimal concentration of the *M. oleifera* whole leaf extract for ethanol-based solvents (pure ethanolic and hydro-ethanolic) ranged between 1 to 20 µg, with the largest zone of inhibition on *E. coli* growth exhibited by the pure ethanol in a dose-dependent manner (Figure 4).

The inhibitory effect of the pure ethanol extract was so potent that we were unable to determine the minimum inhibitory concentration of the solvent even at 1 µg, which prompted us to dilute the extracts even further (Table 3). The minimum inhibitory and minimum bactericidal concentrations for the pure ethanol extract were found to be 0.75 and 3 µg/mL, respectively, whereas, those of the hydo-ethanolic extract were 1.5 and 7-8 µg/mL, respectively (Table 3). The inhibitory and bactericidal concentrations for the other solvents could not be determined even at greater than 20 µg/mL of the extracts (Table 3).

*M. oleifera Extracts Inhibit E. coli Growth at Comparable Masses to Commonly Prescribed Antibiotics:*

The extent to which the 5 different *M. oleifera* leaf extracts inhibited bacterial growth were then compared to antibiotics commonly prescribed for *E. coli* infection (Ciprofloxacin and tetracycline) at comparable masses (5 µg) using diffusion assays. Clindamycin (5 µg) was used as a negative control and showed no inhibition on *E. coli* growth. Both ethanol-based extracts exerted inhibition zones at 5 µg, with the ethanol extract having stronger inhibitory effects when compared to hydro-ethanolic extract (Figure 5). Also, both tetracycline and ciprofloxacin antibiotics exerted inhibition at 5 µg, with ciprofloxacin exhibiting the largest inhibition zone at 35 mm (Figure 5). The ethanol extract had the second largest inhibition zone at approximately 65% that of ciprofloxacin (Figure 5). It was interesting to note that the hydro-ethanolic extract had a larger zone of inhibition compared to tetracycline, i.e., 18 mm versus 14 mm, respectively (Figure 5).

*Ciprofloxacin is Four Times More Potent in Inhibiting Bacterial Growth than M. oleifera Ethanol Extract:*

Next, we sought to determine the concentration of the ethanolic extract required to produce a comparable inhibition zone to ciprofloxacin at 5µg. Since only the extract from pure ethanol exhibited the largest inhibition zone in preceding experiments, it was the only extract tested in subsequent experiments. Data revealed that at a mass of 25µg the pure ethanolic extract exerted an inhibition zone that was comparable to ciprofloxacin at 5µg, implying that the antibiotic was 4-fold more potent than the extract (Table 4).

However, the inhibition zones of the pure ethanolic extract at 25µg on average tended to be slightly larger, by approximately 4-5 mm, than those produced by ciprofloxacin at 5µg (Figure 6). This shows that the inhibition zone of the ethanol extract at 25µg was approximately 120% that of ciprofloxacin (Table 4).

*M. oleifera Dose-dependently Disrupts the Growth Kinetics of E. coli:*

Next, we carried out experiments using varying concentrations (0.75 µg/mL, 1.5 µg/mL and 3 µg/mL) of *Moringa* ethanolic extracts over a period of 72 hours (h), with the goal of examining the effects of *M. oleifera* on the growth kinetics of *E. coli*. The three different concentrations of the ethanolic extract were compared to the positive (only bacteria, no extract) and negative (only extract, no bacteria) control groups (Figure 7). The ethanolic extracts were found to change the growth kinetics of *E. coli* when compared to both controls (Figure 7). Specifically, at 3 µg/mL of ethanolic extract, a concentration considered as the MBC, there was zero growth noted throughout the different time points, up to a total of 72h treatment period (Figure 7). At 1.5 µg/mL of ethanolic extract, a delay in growth lasting 14h was noted, before the onset of exponential growth phase, maximum growth was only 2/3 compared to the positive control and total lysis was observed after 72 h (Figure 7). At 0.75

μg/mL of ethanolic extract a similar delay to the 3μg extract was observed. However, the overall growth was very similar to that of the positive control (Figure 7).

*Comparison of Lethal Dose 50% of Ethanolic M. oleifera Extract Versus Common Antibiotics:*

We then conducted $LD_{50}$ experiments with varying concentrations of the ethanol *M. oleifera* extract and compared them to those of select antibiotics against *E. coli* at 24 hours (Table 5). The $LD_{50}$ for pure ethanol *M. oleifera* extract against *E. coli* was found to be between 2-2.5 μg/mL (Table 4). Streptomycin was found to have the lowest $LD_{50}$ at 4-5 μg/mL (Table 5), and those of tetracycline and ampicillin were 7.5 and 12.5 μg/mL, respectively (Table 5).

*Effects of Select Antibiotics on the Growth Kinetic Patterns of E. coli:*

Next, we conducted experiments that examined the effects of select antibiotics (Ampicillin, streptomycin and tetracycline) on the growth kinetics patterns of *E. coli* at varying concentrations up to 72 hours and at three different concentrations for each antibiotic (1/2 $LD_{50}$, $LD_{50}$, and 2x $LD_{50}$). These antibiotics were compared to positive (only bacteria, no antibiotic) and negative controls (only antibiotic, no bacteria), as described earlier for *M. oleifera* above.

Ampicillin was only found to change the growth kinetics of *E. coli* at two time points when compared to controls (Figure 8). Specifically, at the concentration of ½ $LD_{50}$, $LD_{50}$, and 2x $LD_{50}$, ampicillin was able to reduce growth at the 8h time point when compared to the positive control (Figure 8) and, at 2x $LD_{50}$, ampicillin showed inhibition of *E. coli* growth at the 16h time point when compared to the positive control (Figure 8). Ampicillin was not able to show any

other significant changes in the growth of *E. coli* at any other time points, compared to the positive control (Figure 8).

Streptomycin was found to change the growth kinetics of *E. coli* when compared to controls (Figure 9). Specifically, at the concentrations of ½ $LD_{50}$, $LD_{50}$ and 2x the $LD_{50}$, streptomycin was able to reduce growth observed at the 8h, 16h, and 24h time points when compared to the positive control (Figure 9). Whereas, at 2x the $LD_{50}$ streptomycin concentration also showed reduced levels of *E. coli* growth at 48h and 72h time points, when compared to the positive control (Figure 9).

Tetracycline was able to change the growth kinetics of *E. coli* when compared to the control groups (Figure 10). Specifically, at the concentration of ½ $LD_{50}$, $LD_{50}$, and 2x $LD_{50}$, tetracycline was able to extend the lag phase, and reduce overall growth when compared to the positive control (Figure 10). At the $LD_{50}$ and 2x the $LD_{50}$ tetracycline concentration a difference in *E. coli* growth was observed at all time-points when compared to the positive control (Figure 10). The greatest difference in *E. coli* growth between tetracycline and positive control was noted at 2x $LD_{50}$ (Figure 10). Also, a notable difference in *E. coli* growth was observed after 16h at ½ $LD_{50}$ concentration of tetracycline, when compared to the positive control (Figure 10).

*Proteomics Analysis Reveals that M. oleifera Alters Multiple Biological Processes and Protein Expression:*

Proteomics analysis was utilized to see the change in protein expression observed when *E. coli* was treated with ethanolic *M. oleifera* whole leaf extract, with the goal of delineating underlying mechanisms of action. Proteomics analysis revealed that 64 identifiable proteins in *E. coli* were modulated by *M. oleifera* compared to the control (no treatment) group (Figure 11).

These 64 proteins were found to have many different biological functions and only a small subset, 13 proteins, were found to be significantly changed (Table 6).

The modulated proteins identified during the analysis of the proteomics data were placed into biological processes that were affected by their primary function. The biological processes included: stress response, metabolism, translation, ribosome synthesis, membrane synthesis, ATP synthesis, ion binding, protein synthesis, DNA binding, and periplasmic-binding (Other) (Figure 12).

Based upon a protein's primary function within a cell, two biological processes, metabolism and stress response were found to be affected the most by the ethanolic *M. oleifera* whole leaf extract treatment (Figure 12). Proteomics data showed17 proteins involved in metabolism that changed, accounting for 26% of the overall change in variable protein expression observed (Figure 12). There were 20 proteins involved in stress response that changed, accounting for 31% of the overall change in variable protein expression observed (Figure 12). Metabolism and stress response biological processes accounted for 57% of the overall change in variable protein expression. The remaining 8 biological processes had ≤ 5 (8%) proteins changed in each process, accounting for 43% of the overall change in variable protein expression (Figure 12).

*Quantitative Proteomics Expression Analysis of Selected Significantly Changed Stress Response Proteins:*

Significantly changed stress response proteins were selected for quantitative expression analysis including isocitrate dehydrogenase [NADP] (IDH), DNA-binding protein HU-alpha, ornithine carbamoyltransferase chain F, and alcohol dehydrogenase propanol-preferring (ADHP) (Figure 13). Selected stress response proteins were found to have significantly

increased expression for 3 of 4 proteins when *E. coli* cells were treated with ethanolic *M. oleifera* whole leaf extract (Figure 13). DNA-binding protein HU-alpha was the only selected stress response protein that had a significant decrease in expression, approximately 35%, when *E. coli* cells were treated with ethanolic *M. oleifera* whole leaf extract (Figure 13). IDH was found to have an increase of approximately 2-fold between control (no treatment) and ethanolic *Moringa* treated *E. coli* cells (Figure 13). Ornithine carbamoyltransferase chain F was found to have an increase of approximately 6.5-fold between control (no treatment) and ethanolic *Moringa* treated *E. coli* cells (Figure 13). ADHP was found to have an increase of approximately 3-fold between control (no treatment) and ethanolic *Moringa* treated *E. coli* cells (Figure 13).

*Quantitative Proteomics Expression Analysis of Selected Significantly Changed Proteins Involved in Metabolism:*

Significantly changed proteins involved in metabolism were selected for quantitative expression analysis including uncharacterized oxidoreductase YajO, and malate dehydrogenase (MDH) (Figure 14). Selected proteins involved in metabolism were found to have significantly increased expression for both selected proteins when *E. coli* cells were treated with ethanolic *M. oleifera* whole leaf extract (Figure 14). Uncharacterized oxidoreductase YajO was found to have an increase of approximately 2-fold between control (no treatment) and ethanolic *Moringa* treated *E. coli* cells (Figure 14). MDH was found to have an increase of approximately 2.25-fold between control (no treatment) and ethanolic *Moringa* treated *E. coli* cells (Figure 14).

*Quantitative Proteomics Expression Analysis of Selected Significantly Changed Proteins Involved in ATP Synthesis:*

Significantly changed proteins involved in ATP synthesis were selected for quantitative expression analysis including ATP synthase subunit c, Succinyl-CoA ligase subunit beta (SUCC), and Succinyl-CoA ligase subunit alpha (SUCD) (Figure 15). Selected proteins involved in ATP synthesis were found to have significantly increased expression for all three selected proteins when *E. coli* cells were treated with ethanolic *M. oleifera* whole leaf extract (Figure 15). ATP synthase subunit c was found to have an increase in expression of approximately 2-fold between control (no treatment) and ethanolic *Moringa* treated *E. coli* cells (Figure 15). SUCC was found to have an increase in expression of approximately 2-fold between control (no treatment) and ethanolic *Moringa* treated *E. coli* cells (Figure 15). SUCD was found to have an increase in expression of approximately 2.25-fold between control (no treatment) and ethanolic *Moringa* treated *E. coli* cells (Figure 15).

*Quantitative Proteomics Expression Analysis of Selected Significantly Changed Proteins Involved in Translation, Ribosome Synthesis, Membrane Synthesis, and Periplasmic-binding:*

Significantly changed proteins involved in translation, ribosome synthesis, membrane synthesis, and periplasmic-binding were selected for quantitative expression analysis including 50S ribosomal L5 for translation, ribosomal large subunit pseudouridine synthase B as the protein involved in ribosome synthesis, Enoyl-[acyl-carrier-protein] reductase [NADH] FabI as a protein involved in membrane synthesis, and glutamate/aspartate periplasmic-binding protein as a periplasmic-binding functioning protein (Figure 16). Selected proteins involved in ribosome synthesis, membrane synthesis, and periplasmic-binding were found to have significantly increased expression for all three selected proteins when *E. coli* cells were treated with ethanolic *M. oleifera* whole leaf extract (Figure 16). The selected protein involved in

translation was found to have a significant decrease in expression for the selected protein when *E. coli* cells were treated with ethanolic *M. oleifera* whole leaf extract (Figure 16). The protein involved in ribosome synthesis, ribosomal large subunit pseudouridine synthase B, was found to have an increase in expression of approximately 2-fold between control (no treatment) and ethanolic *Moringa* treated *E. coli* cells (Figure 16). The protein involved in membrane synthesis, Enoyl-[acyl-carrier-protein] reductase [NADH] FabI, was found to have an increase in expression of approximately 2-fold between control (no treatment) and ethanolic *Moringa* treated *E. coli* cells (Figure 16). The periplasmic-binding functioning protein, glutamate/aspartate periplasmic-binding protein, was found to have an increase in expression of approximately 3-fold between control (no treatment) and ethanolic *Moringa* treated *E. coli* cells (Figure 16). The protein involved in translation, 50S ribosomal protein L5, was found to have a decrease in expression of approximately 40% between control (no treatment) and ethanolic *Moringa* treated *E. coli* cells (Figure 16).

## Discussion

The present study uses *in vitro* techniques to examine the biological effects of *M. oleifera* on *E. coli*. The overall objective of the present study was to characterize the anti-bacterial properties as well as underlying mechanism of action of *M. oleifera in vitro*, with the long-term goal of testing the effectiveness of *M. oleifera* in preventing preterm labor. Specifically, we screened different whole leaf extracts of *M. oleifera* for anti-bacterial activities and bacterial growth compared to commonly used antibiotics using various *in vitro* assays. Only the ethanol-based extracts (pure ethanolic and hydro-ethanolic) was found to exert significant anti-bacterial activity against *E. coli.* These data confirms earlier findings on the anti-bacterial properties of *M. oleifera* and highlights its potential use as a natural anti-bacterial agent for the management and prevention of infection-induced preterm labor and other disorders.

The medicinal activities of *M. oleifera* have been widely reported, including anti-bacterial properties (Anwar, Latif, Ashraf, & Gilani, 2007; Colicchio et al., 2015; Dewangan et al., 2010; Mbikay, 2012; Pal, Mukherjee, & Saha, 1995; Sreelatha, Jeyachitra, & Padma, 2011), and, notably, its inhibitory effects on *E. coli* growth using extracts of different solvents and parts of the plant (Auwal et al., 2013; Ekundina, Ebeye, Oladele, & Osham, 2015; Thilza et al., 2010). In the present study, of the five different solvents (water, methanol, butanol, ethanol, and a hydro-ethanolic, 80/20) used to extract phytochemicals from *Moringa* leaves, only the ethanol-based extracts (ethanolic and hydro-ethanolic) were able to inhibit *E. coli* growth. These current observations are consistent with earlier microbial studies that used ethanol-based extracts, including earlier *E. coli* studies (Adline & Devi, 2014; Bukar et al., 2010; Lar, Ojile, Dashe, & Oluoma, 2011). Previous HPLC studies have examined the various phytochemical compounds

from seeds and leaves using various solvent extracts, including ethanol and methanol (Bennett et al., 2003; Fahey, 2005; Siddhuraju & Becker, 2003). Specifically, phytochemical compounds isolated using ethanol and methanol solvents include pterygospermin, quercetin-3-O-glucoside and quercetin-3-O-(6''- malonylglucoside), kaempferol-3-Oglucoside and kaempferol-3-O-(6''-malonyl-glucoside), 3-caffeoylquinic acid, 5-caffeoylquinic acid, 4- (4'-O-acetyl-a-L-rhamno pyranosyloxy) benzyl isothiocyanate, 4-(a-L-rhamnopyranosyloxy) benzyl isothio cyanate, niazimicin, benzyl isothiocyanate, and 4-(a-L-rhamnopyranosyloxy) benzyl glucosinolate. Most of these bioactive compounds appear to play a role in both anti-bacterial and anti-inflammatory properties, making *M. oleifera* an ideal treatment for both infection- and or inflammatory-induced preterm labor (Bennett et al., 2003; El Sohaimy, Hamad, Mohamed, Amar, & Al-Hindi, 2015; Fahey, 2005; Siddhuraju & Becker, 2003). It appears that the phytochemical activity of the whole ethanolic leaf extract of *M. oleifera* is better suited for inhibiting bacteria in contrast to the methanolic leaf extract, whose phytochemical properties appear to have a higher potency for inhibiting inflammation and or cancer (Rao, Devi, & Kamath, 2001; Stohs & Hartman, 2015; Verma, Vijayakumar, Mathela, & Rao, 2009). However, the full list of bioactive compounds in the ethanol-based leaf extracts for *Moringa* is incomplete and ongoing studies in our labs are currently characterizing a comprehensive list of the compounds, their chemical structures and biological activities.

The present data generated from experiments that compared the potency of the ethanolic extract of *M. oleifera* whole leaf to inhibit *E. coli* growth versus commonly prescribed antibiotics were insightful and promising, particularly in view of the ever growing resistance to a broad range of antibiotics. We found that the ethanolic extract of *M. oleifera* whole leaf extract significantly inhibited *E. coli* growth more than selected antibiotics, specifically ampicillin and clindamycin at comparable concentrations. Whereas the ethanolic extract of *M. oleifera* whole leaf significantly inhibited *E. coli* growth comparable to the antibiotics, specifically tetracycline

and streptomycin at comparable concentrations. However, ciprofloxacin was found to be 4-fold more potent than *M. oleifera* and had a greater potency in inhibiting *E. coli* growth. These current findings are consistent with an earlier report (Kumar, Pandey, Mohan, & Singh, 2012), although some other previous studies have shown that commonly prescribed antibiotics, such as tetracycline and ciprofloxacin, inhibit *E. coli* at lower concentrations when compared to whole leaf extracts from *M. oleifera* (Kumar et al., 2012; Thilza et al., 2010), thus contradicting the present data. The cause of discrepancy between the present data and these earlier reports are not yet clear but could be because of the difference in the solvent and parts of the plant used for extraction, i.e., the earlier studies used a combination of water and leaf stalk, and not ethanol and whole leaf used here (Thilza et al., 2010). There is need for a more comprehensive anti-bacterial study that will compare the different activities of various plant parts and solvent extracts in a broad range of bacterial species (Kumar et al., 2012).

Since both *Moringa* and the select antibiotics used in the present study exhibited similar effects on *E. coli*, including growth inhibition and kinetic changes, it is likely that there perhaps some common pathways that likely mediate the effects of the plant and pharmaceuticals, most of which have been extensively studied for ciprofloxacin, tetracycline, streptomycin, ampicillin and clindamycin (Hermann, 2007; Kohanski, Dwyer, & Collins, 2010; Rafailidis, Ioannidou, & Falagas, 2007; Sharma, Jain, Pahwa, & Yar, 2010). For instance, ciprofloxacin targets DNA gyrase, leading to the disrupting of replication and transcription and subsequently in DNA fragmentation and cellular death (Sharma et al., 2010). On the other hand, tetracycline kills the bacteria by binding to the bacterial 30S ribosomal subunit near the A site, which consequently inhibits protein synthesis through the prevention tRNA docking (Zakeri & Wright, 2008). Streptomycin interferes with translation by binding to a specific site found on 16S subunit on the ribosome with three adenine residues, which is responsible for decoding mRNA (Hermann, 2007). This interference, coupled with translation leads to the accumulation of non-functional,

incorrectly folded, and in-effective as well as in-efficient proteins that lead to bacterial cell death (Hermann, 2007). Ampicillin, like other penicillin-like antibiotics, is able to act in a bactericidal manner by inhibiting and preventing the production of the major cell wall component peptidoglycan; the bacterial cell ultimately undergoes cell lysis due to the inhibition of cell wall synthesis (Rafailidis et al., 2007). Clindamycin, the last common antibiotic used to compare to *M. oleifera*, binds to the 23s portion of the 50S subunit of bacterial ribosomes and cause premature dissociation of the peptidyl-tRNA from the ribosome, which leads to the inhibition of protein synthesis (Kohanski et al., 2010).

Overall, it appears that all of these key antibiotics against *E. coli* commonly target key cellular processes, such as replication, transcription, protein synthesis, and cell wall synthesis. For now, it appears that the ethanolic *M. oleifera* whole leaf extract inhibits *E. coli* in a similar manner as the key antibiotics, according to analysis of proteomics data, except *M. oleifera* appears to disrupt multiple biological processes including stress response, metabolism, ribosome synthesis, membrane synthesis, and ATP synthesis. It is also interesting to note that kinetic patterns show that *M. oleifera* inhibit *E. coli* during the different phases of growth at different concentrations of the whole leaf extract, a trend that is very similar to that of common antibiotics, such as streptomycin, tetracycline and ampicillin. Surprisingly, most of the common antibiotics were less effective at inhibiting *E. coli* growth at comparable concentrations to *Moringa*. Indeed, in the present study, the ethanolic extract of *M. oleifera* was able to elicit more pronounced changes to the different growth phases of *E. coli* when compared to tetracycline, streptomycin and ampicillin. The phases most affected by the ethanolic extract of *M. oleifera* whole leaf extract in the present study were the lag, exponential and death phases, within the normal kinetic growth pattern of *E. coli*. The ethanolic extract of *M. oleifera* was also able to prolong the lag phase, and at 1.5 μg/mL , increased the effects of the death phase, leading to complete lysis of *E. coli*.

The exact mechanism through which the ethanolic- and hydroethanolic-extracted bioactive compounds exert anti-*E. coli* properties also remain unclear. Preliminary proteomics data shows that ethanolic *M. oleifera* whole leaf extract alters expression of more than 64 proteins involved in various biological processes of *E. coli,* with 13 of the proteins being significantly changed. These biological processes are varied and include the stress response, metabolic processes, translation, ribosomal synthesis, membrane synthesis, ATP synthesis, and other biological processes.

Stress response proteins identified included isocitrate dehydrogenase [NADP] (IDH), DNA-binding protein HU-alpha, ornithine carbamoyltransferase chain F, and alcohol dehydrogenase propanol-preferring (ADHP) (Figure 13). IDH functions enzymatically in the Kreb's cycle by performing oxidative decarboxylation of isocitrate and is regulated by phosphorylation and de-phosphorylation (LaPorte, 1993). Regulation of this enzyme only occurs when ATP levels are sufficient and this is done by isocitrate dehydrogenase kinase/phosphatase (Taylor, Hu, Hart, & McAlister-Henn, 2008). The increased expression of this protein in treated ethanolic *M. oleifera* whole leaf extract *E. coli* cells can be indicative of oxidative stress, improper protein function, and improper Kreb's cycle regulation (Krisko, Copic, Gabaldon, Lehner, & Supek, 2014). Another protein identified by proteomics data was DNA-binding protein HU-alpha which is a protein involved in protecting the DNA during stressful environments (Prieto et al., 2011). However, DNA-binding protein HU-alpha is down-regulated during ethanolic *M. oleifera* treatment in *E. coli* which could indicate fragmentation has already occurred, improper packaging of the DNA, or the cells inability to transcribe the DNA-protecting protein when compared to control (Khil & Camerini-Otero, 2002). Ornithine carbamoyltransferase chain F was another protein involved in stress response with the greatest enhancement of 6.5-fold by ethanolic *M. oleifera* treatment of *E. coli* according to the proteomics data (Figure 13). This protein has been found to be up-regulated during treatments where

DNA-damage is occurring and is involved in overall amino-acid biosynthesis in *E. coli* and other pathogenic organisms such as *Staphylococcus aureus* (Alves et al., 2014; Khil & Camerini-Otero, 2002). Alcohol dehydrogenase propanol-preferring (ADHP) was the last selected stress response protein to be enhanced by ethanolic *M. oleifera extract* treatment according to the proteomics data (Figure 13). ADHP functions in breaking down propanol but is enhanced during the presence of DNA damaging stimulus, as well as acetaldehyde catabolic processes (Khil & Camerini-Otero, 2002). All of these proteins in *E. coli* treated with ethanolic *M. oleifera* whole leaf extract depict a stress response towards DNA damage, metabolic stress, and cell death.

Proteins involved in metabolism identified during proteomics analysis included oxidoreductase YajO, and malate dehydrogenase (MDH) (Figure 14). Oxidoreductase YajO was enhanced by 2-fold by ethanolic *M. oleifera* treatment in *E. coli* and functions in thiamine metabolism through oxidation-reduction processes (Gagliardi et al., 2016). MDH was another protein involved in metabolism and was enhanced by 2.25-fold by ethanolic *M. oleifera* treatment in *E. coli* and functions in oxidation-reduction reactions of malate to oxaloacetate during fermentation and gluconeogenesis (Sutherland & McAlister-Henn, 1985; Takahashi-Iniguez, Aburto-Rodriguez, Vilchis-Gonzalez, & Flores, 2016). The enhancement of these two proteins by ethanolic *M. oleifera* extract in *E. coli* could potentially mean the bacterium is having trouble efficiently metabolizing and is being restricted to fermentation and producing its own glucose while scavenging for other carbon sources (Gagliardi et al., 2016; Takahashi-Iniguez et al., 2016). All of these proteins in *E. coli* treated with ethanolic *M. oleifera* whole leaf extract depict metabolic restriction with a stress response from oxidative metabolism to anaerobic metabolism.

Specific ATP-synthesizing proteins identified during proteomics analysis included ATP synthase subunit c, Succinyl-CoA ligase subunit beta (SUCC), and Succinyl-CoA ligase subunit

alpha (SUCD) (see Figure 15). All of these proteins are involved in ATP synthesis and are enhanced 2-2.25-fold in *E. coli* cells treated with ethanolic *M. oleifera* whole leaf extract (see figure 15). ATP synthase subunit c was enhanced 2-fold according to the proteomics data and is involved in ATP synthesis by utilizing proton transport across the membrane, and used to re-establish the proton gradient across the membrane through ATP hydrolysis (Capaldi, Schulenberg, Murray, & Aggeler, 2000; Deckers-Hebestreit, Greie, Stalz, & Altendorf, 2000). SUCC and SUCD were also enhanced, 2-fold and 2.25-fold respectively, according to the proteomics data and are involved in energy synthesis during aerobic and anaerobic growth (Reed, Vo, Schilling, & Palsson, 2003). SUCC, and SUCD are likely enhanced during ethanolic *M. oleifera* whole leaf extract in *E. coli* because of the potential fermentation/anaerobic reactions needed for survival and these proteins have the capability of producing energy in substrate limiting environments by providing succinyl-coA for anaerobic growth when oxidative growth is hindered (Capaldi et al., 2000; Goo, An, Kang, & Hwang, 2015; Shimizu, 2013). All of these proteins in *E. coli* treated with ethanolic *M. oleifera* whole leaf extract depict membrane potential issues, and energy inefficiency due to a potential substrate limiting environment with an energy adaptation from aerobic growth to anaerobic growth.

A variety of proteins identified during proteomics analysis were involved in biological processes such as translation (50S ribosomal protein L5), ribosome synthesis (Ribosomal large subunit pseudo uridine synthase B), membrane synthesis (Enoyl-[acyl-carrier-protein] reductase [NADH] FabI), and periplasmic-binding (glutamate/aspartate periplasmic-binding protein) (see Figure 16). 50S ribosomal protein L5 was down-regulated in *E. coli* treated with ethanolic *M. oleifera* whole leaf extract and functions in assisting 5S RNA attachment to large ribosomal subunit (Chen & Williamson, 2013; Spierer & Zimmermann, 1978). We speculate that translation will be inefficient due to the down-regulation of 50S ribosomal protein L5 and the lack of RNA attachment to the large ribosomal subunit which can lead to accumulation of

faulty large ribosomal subunits in *E. coli* treated with ethanolic *M. oleifera* whole leaf extract (Chen & Williamson, 2013; Korepanov et al., 2012). Ribosomal large subunit pseudouridine synthase B was enhanced by 2-fold according to the proteomics data and is involved in pseudouridine synthase (Conrad, Sun, Englund, & Ofengand, 1998; Del Campo, Kaya, & Ofengand, 2001). Pseudouridine is a key component in ribosomes and is needed for normal growth and ribosome function (Charette & Gray, 2000; Del Campo et al., 2001). Pseudoruridine is seen as a post-transcriptional modification and has been postulated to be a piece of an adaptive mechanism required in *E. coli* to survive in environmental stresses such as that placed upon it by the ethanolic *M. oleifera* whole leaf extract treatment (Conrad et al., 1998; Karijolich, Yi, & Yu, 2015). Enoyl-[acyl-carrier-protein] reductase [NADH] FabI was a protein enhanced by 2-fold in *E. coli* according to proteomics data and is involved in fatty-acid biosynthesis, lipid metabolism, biotin biosynthesis, and has been associated with responses to antibiotics (Bergler et al., 1994; Chan & Vogel, 2010). The enhancement of this lipid synthesizing protein could be due to the bacterial cell trying to keep its membrane structure intact and membrane potential stable. The periplasmic-binding functioning protein glutamate/aspartate periplasmic-binding protein was also enhanced 3-fold according to proteomics data (see Figure 16). This periplasmic-binding functioning protein could be responsible for chemotaxis, transmembrane transport, and could play a role in dictating transcription based upon its evolutionary origin with a class of DNA-binding proteins (Aguilera et al., 2014; Tam & Saier Jr., 1993).

Based on the already known activities of some of the identified clusters of bioactive compounds contained in the *M. oleifera* ethanol extracts, pterygospermin appears to inhibit the transaminase enzyme and causes membrane disruption in bacteria cells (Onyekaba, Omojate, & Anowi, 2013); Tannins, which are polyphenols, possess potent anti-bacterial proliferation activities exerted by blocking essential enzymes involved in microbial metabolism, such as the proteolytic macerating enzymes (Moyo, Masika, & Muchenje, 2012); Saponins induce

bacteriolysis by eliciting fatal structural changes in the cell membrane (Omojate, Enwa, Jewo, & Eze, 2014); Further, polyphenols, such as gallic acids likely act by binding to bacterial dihydrofolate reductase (DHFR) enzymes, subsequently inhibiting the supercoiling activity of *E.coli* bacterial gyrase via binding to the ATP binding site of gyrase B and to bacterial DNA, ultimately inducing topoisomerase IV enzyme-mediated DNA cleavage and bacterial growth stasis (Jayaraman, Sakharkar, Lim, Tang, & Sakharkar, 2010; Omojate et al., 2014). Ethanolic *M. oleifera* whole leaf extract appears to inhibit similar biological processes as the HPLC-isolated bioactive compounds in *E.coli* based on the analysis of current proteomics data.

Although the exact reasons for this apparent advantage of *Moringa* over pharmaceuticals is for now unclear, it could be attributed to the presence of a cocktail of anti-bacterial phytochemicals in the plant, which enables it to manipulate multiple biological processes of the bacterial synergistically. Indeed, in support of this speculation, our preliminary proteomics data shows that the plant alters expression of more than 64 proteins involved in various biological processes of *E. coli,* with 13 of the proteins being significantly changed. These biological processes are varied and include stress response, metabolism, translation, ribosomal synthesis, membrane synthesis, ATP synthesis, and other biological processes. Future studies in our lab will verify these proteomics data and characterize the specific bioactive compounds inducing these biological changes and their underlying signaling pathways.

For now, we do not know the specific target *signature* molecules (RNA/protein) that lead to the demise of the bacteria observed here as bacterial inhibition, delayed growth and structural changes. The mechanism of action by *M. oleifera* to cause these structural effects is probably multi-factorial through targeting many different cellular processes in the bacteria resulting in the structural changes reported in the present study, as revealed by the proteomics data. According to the data gathered, along with what has already been proposed previously we propose a working model which shows ethanolic *M. oleifera* inhibiting such processes as

ribosome synthesis, and translation; but increasing processes such as oxidative stress, membrane instability, DNA packaging, DNA fragmentation and DNA lysis in *E. coli* which will eventually culminate in lysis of the *E. coli* cell (see Figure 17). Future studies should investigate trends in increased concentrations of extract on these processes.

Premature births are largely caused by bacterial infections, notably *E. coli*, one of the main causes implicated in infections associated with preterm labor. The growing resistance of bacteria to the current drugs of choice, and the possible associated side effects of antibiotic use, demands an urgent development of safer but effective alternatives. We demonstrate here that pure ethanolic and hydro-ethanolic (80/20) *M. oleifera* extracts exert potent bactericidal properties against the bacteria *E. coli* that is greater than the other extracts and are comparable to antibiotics of choice for this microbe. We also demonstrate the phases of growth of *E. coli* affected by the ethanolic *M. oleifera* whole leaf extract and compared these effects to common antibiotics. These present data suggest a potential use of this medicinal plant in preventing *E. coli*-induced preterm labor, without the side effects associated with antibiotics. Going forward, there is need to conduct a more comprehensive study that will: **a**) identify the specific bioactive compounds inhibiting *E. coli* growth in whole leaf extract, **b**) compare the potency of whole leaf to the other parts of the plants, **c**) compare other solvents such as acetone, petroleum ether, ethyl acetate, and citrus terpenes; **d**) define the underlying mechanisms by which the extract inhibits bacterial growth; and **e**) test whether *M. oleifera* can inhibit preterm labor in animal models of preterm labor by blocking pathogenic bacterial strains. Ongoing studies in our lab are currently screening clusters of HPLC-isolated bioactive compounds, verifying proteomics data and delineating the underlying signaling pathways and mechanisms induced by whole leaf *Moringa*.

In summary, our data supports the current thinking in the field of medicinal plants, that *Moringa oleifera* whole-leaf ethanolic extract has antibacterial properties. We also provide the

first expression profile for significantly changed proteins when *E. coli* is incubated with ethanolic *M. oleifera* extract and their pattern of expression to various biological processes revealed by proteomics. From all the data gathered in the present study, we propose a working model for ethanolic *M. oleifera* whole leaf extract's inhibition of *E. coli*. Future studies will assess *M. oleifera's* antibacterial properties against various other bacteria, including pathogenic *E. coli* strains, and whether the significantly changed proteins observed in the proteomics results are interconnected to specific compounds found in the ethanolic *M. oleifera* extract which results in lysis of the bacteria.

## References


Abraham A. N., & Okon G. O. (2014). Antibacterial effect of *Moringa oleifera* extracts on bacteria associated with urinary tract infection. *International Journal of Research,* 1(8), 1308-1316. Retrieved from https://internationaljournalofresearch.com

Adib-Conquy, M., Scott-Algara, D., Cavaillon, J., & Souza-Fonseca-Guimaraes, F. (2014). TLR-mediated activation of NK cells and their role in bacterial/viral immune responses in mammals. *Immunology and Cell Biology*, 92(3), 256-262. doi: 10.1038/icb.2013.99

Adline, J., & Devi, A. (2014). A study on phytochemical screening and antibacterial activity of *Moringa oleifera*. *International Journal of Research in Applied, Natural and Social Sciences,* 2(5), 169-176.

Agrawal, V., & Hirsch, E. (2012). Intrauterine Infection and preterm labor. *Seminars in Fetal and Neonatal Medicine,* 17(1), 12-19. doi: 10.1016/j.siny.2011.09.001

Aguilera, L., Toloza, L., Gimenez, R., Odena, A., Oliveira, E., Aguilar, J., Badia, J., & Baldoma, L. (2014). Proteomic analysis of outer membrane vesicles from the probiotic strain *Escherichia coli* Nissile 1917. *Proteomics,* 14(2-3), 222-229. doi: 10.1002/pmic.201300328

Alves, E., Faustino, M., Neves, M., Cunha, A., Tome, J., & Almeidaa, A. (2014). An insight on bacterial cellular targets of photodynamic inactivation. *Future Medicinal Chemistry,* 6(2), 141-164. doi: 10.4155/fmc.13.211

Amelo, W., Nagpal, P., & Makonnen, E. (2014). Antiplasmodial activity of solvent fractions of methanolic root extract of *Dodonaea angustifolia* in *Plasmodium berghei* infected mice. *BMC Complemententary and Alternative Medicine,* 14(462), 462. doi: 10.1186/1472-6882-14-462.

Anwar, F., Latif, S., Ashraf, M., & Gilani, A. H. (2007). *Moringa oleifera*: A food plant with multiple medicinal uses. *Phytotherapy Research,* 21(1), 17-25. doi: 10.1002/ptr.2023

Auwal, M. S., Tijjani, A. N., Sadiq, M. A., Saka, S., Mairiga, I. A., Shuaibu, A., Adawaren, E., & Gulani, I. A. (2013). Antibacterial and haematological acitivty of *Moringa oleifera* aqueous seed extract in Wistar albino rats. *Sokoto Journal of Veterinary Sciences,* 11(1), 28-37. Retrieved from http://www.ajol.info/index.php/sokjvs/article/view/89890/79345

Beck, S., Wojdyla, D., Say, L., Betran, A. P., Merialdi, M., Requejo, J. H., Rubens, C., Menon, R., & Van Look, P. A. (2009). The world-wide incidence of preterm births: a systematic review of maternal mortality and morbidity. *Bulletin of the World Health Organization,* 88, 31-38. doi: 10.2471/BLT.08.062554.

Bennett, R., Mellon, F., Foidl, N., Pratt, J., Dupont, M., Perkins, L., & Kroon, P. (2003). Profiling Glucosinolates and Phenolics in Vegetative and Reproductive Tissues of the Multi-purpose Trees *Moringa oleifera L.* (Horseradish Tree) and *Moringa stenopetala L. Journal of Agricultural and Food Chemistry,* 51(12), 3546-3553. doi: 10.1021/jf0211480

Bergler, H., Wallner, P., Ebeling, A., Leitinger, B., Fuchsbichler, S., Aschauer, H., Kollenz, G., Hoegenauer, G., & Turnowsky, F. (1994). Protein EnvM is the NADH-dependent enoyl-ACP reductase (FabI) of *Escherichia coli. Journal of Biological Chemistry,* 269(8), 5493-5496. Retrieved from http://www.jbc.org/content/269/8/5493.long

Blair, J., Webber, M., Baylay, A., Ogbolu, D., & Piddock, L. (2015). Molecular mechanisms of antibiotic resistance. *Nature Reviews Microbiology,* 13, 42-51. doi: 10.1038/nrmicro3380

Blencowe, H., Cousens, S., Oestergaard, M., Chou, D., Moller, A. B., Narwal, R., Adler, A., Garcia, C. V., Rohde, S., Say, L., & Lawn, J. E. (2012). National, regional and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. *The Lancet*, 379(9832), 2162-2172. doi: 10.1016/S0140-6736(12)60820-4

Bukar, A., Uba, A., & Oyeyi, T. I. (2010). Antimicrobial Profile of *Moringa oleifera Lam.* extracts against some food-borne microorganisms. *Bayero Journal of Pure and Applied Sciences,* 3(1), 43-48. Retrieved from http://www.ajol.info/index.php/bajopas/article/view/58706

Capaldi, R. A., Schulenberg, B., Murray, J., & Aggeler, R. (2000). Cross-linking and electron microscopy studies of the structure and functioning of the *Escherichia coli* ATP synthase. *Journal of Experimental Biology,* 203(Pt 1), 29-33. Retrieved from http://jeb.biologists.org/content/203/1/29.long

Challis, J.R., Lockwood, C. J., Myatt, L., Norman, J. E., Strauss, J. F., & Petraglia, F. (2009). Inflammation and pregnancy. *Reproductive Sciences*, 16(2), 206-215. doi: 10.1177/1933719108329095

Chan, D. I., & Vogel, H. J. (2010). Current understanding of fatty acid biosynthesis and the acyl carrier protein. *Biochemical Journal,* 430(1), 1-19. doi: 10.1042/BJ20100462

Charette, M., & Gray, M. (2000). Pseudouridine in RNA: What, where, how, and why. *International Union of Biochemistry and Molecular Biology Life,* 49(5), 341-351. doi: 10.1080/152165400410182

Charoensin, S. (2014). Antioxidant and anticancer activities of *Moringa oleifera* leaves. *Journal of Medicinal Plants Research,* 8(7), 318-325. doi: 10.5897/JMPR2013.5353

Chen, S. S., & Williamson, J. R. (2013). Characterization of the ribosome biogenesis landscape in *E. coli* using quantitative mass spectrometry. *Journal of Molecular Biology,* 425(4), 767-779. doi: 10.1016/j.jmb.2012.11.040

Colicchio, C., Ohashi, T., Brunson, A., Slobada, C., Emrani, J., Edassi, J., Jesmin, S., & Mowa, C. (2015). *Moringa oleifera's* Whole Methanolic Extract Attenuates Levels of Pro-inflammatory Markers in the Cervix of Preterm Labor Mice Models. *The Federation of American Societies for Experimental Biology Journal,* 29(1), Supplement 721.42. Abstract retrieved from The FASEB Journal.

Conrad, J., Sun, D., Englund, N., & Ofengand, J. (1998). The rluC Gene of Escherichia coli Codes for a Pseudouridine Synthase That Is Solely Responsible for Synthesis of Pseudouridine at Positions

955, 2504, and 2580 in 23 S Ribosomal RNA. *Journal of Biological Chemistry,* 273(29), 18562-18566. Retrieved from http://www.jbc.org/content/273/29/18562.long

Cram, L. F., Zapata, M., Toy, E. C., & Baker, B. (2002). Genitourinary Infections and Their Association with Preterm Labor. *American Family Physician,* 65(2), 241-249. Retrieved from http://www.aafp.org/afp/2002/0115/p241.html

Deckers-Hebestreit, G., Greie, J., Stalz, W., & Altendorf, K. (2000). The ATP synthase of *Escherichia coli*: structure and function of $F_0$ subunits. *Biochimica et Biophysica Acta (BBA) - Bioenergetics,* 1458(2-3), 364-373. doi: 10.1016/S0005-2728(00)00087-6

Del Campo, M., Kaya, Y., & Ofengand, J. (2001). Identification and site of action of the remaining four putative pseudouridine synthases in *Escherichia coli*. *RNA,* 7(11), 1603-1615. Retrieved from http://rnajournal.cshlp.org/content/7/11/1603.long

Dewangan, G., Koley, K., Vadlamudi, V., Mishra, A., Poddar, A., & Hirpurkar, S. (2010). Antibacterial activity of *Moringa oleifera* (drumstick) root bark. *Journal of Chemical and Pharmaceutical Research,* 2(6), 424-428. Retrieved from http://jocpr.com/

Ekundina, V., Ebeye, O., Oladele, A., & Osham, G. (2015). Hepatotoxic and Nephrotoxic Effects of *Moringa oleifera* Leaves Extract in Adult Wistar Rats. *Journal of Natural Sciences Research,* 5(3), 110-117. Retrieved from http://www.iiste.org/Journals/index.php/JNSR/article/view/20080

El-Bastawissi, A. Y., Williams, M. A., Riley, D. E., Hitti, J., & Krieger, J. N. (2000). Amniotic fluid interleukin-6 and preterm delivery: a review. *Obstetrics & Gynecology,* 95(6 pt 2), 1056-64. Retrieved from http://journals.lww.com/greenjournal/toc/2000/06001

El Sohaimy, S., Hamad, G., Mohamed, S., Amar, M., & Al-Hindi, R. (2015). Biochemical and functional properties of *Moringa oleifera* leaves and their potential as a functional food. *Global Advanced Research Journal of Agricultural Science,* 4(4), 188-199. Retrieved from http://garj.org/garjas/index.htm

Fahey, J. W. (2005). *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. *Trees for Life Journal,* 1(5), 1-16. Retrieved from http://www.TFLJournal.org/article.php/20051201124931586

Gagliardi, A., Lamboglia, E., Bianchi, L., Landi, C., Armini, A., Ciolfi, S., Bini, L., & Marri, L. (2016). Proteomics analysis of a long-term survival strain of *Escherichia coli* K-12 exhibiting a growth advantage in stationary-phase (GASP) phenotype. *Proteomics*, 16(6), 963-972. doi: 10.1002/pmic.201500314

Goo, E., An, J., Kang, Y., & Hwang, I. (2015). Control of bacterial metabolism by quorum sensing. *Trends in Microbiology,* 23(9), 567-576. doi: http://dx.doi.org/10.1016/j.tim.2015.05.007

Hermann, T. (2007). Aminoglycoside antibiotics: old drugs and new therapeutic approaches. *Cell and Molecular Life Sciences,* 64(14), 1841-1852. doi: 10.1007/s00018-007-7034-x

Jayaraman, P., Sakharkar, M., Lim, C., Tang, T., & Sakharkar, K. (2010). Activity and interactions of antibiotic and phytochemical combinations against *P. aeruginosa in vitro*. *International Journal of Biological Sciences,* 6(6), 556-568. doi: 10.7150/ijbs.6.556

Karijolich, J., Yi, C., & Yu, Y. (2015). Transcriptome-wide dynamics of RNA pseudouridylation. *Nature Reviews Molecular Cell Biology,* 16(10), 581-585. doi: 10.1038/nrm4040

Kasolo, J. N., Bimenya, G. S., Ojok, L., Ochieng, J., & Ogwal-Okeng, J. W. (2010). Phytochemicals and uses of *Moringa oleifera* leaves in Ugandan rural communities. *Journal of Medicinal Plants Research,* 4(9), 753-757. doi: 10.5897/JMPR10.492

Khil, P. P., & Camerini-Otero, R. D. (2002). Over 1000 genes are involved in the DNA damage response of *Escherichia coli. Molecular Microbiology,* 44(1), 89-105. doi: 10.1046/j.1365-2958.2002.02878.x

Klein, L. L., & Gibbs, R. (2004). Use of microbial cultures and antibiotics in the prevention of infection-associated preterm birth. *American Journal of Obstetrics and Gynecology,* 190(6), 1493-1502. doi: 10.1016/j.ajog.2004.03.014

Kohanski, M., Dwyer, D., & Collins, J. (2010). How antibiotics kill bacteria: from targets to networks. *Nature Reviews Microbiology,* 8(6), 423-435. doi: 10.1038/nrmicro2333

Konda, V., Madhavi, E., Ruckmani, A., & Venkataramana, Y. (2013). A Review on medicinal plants with potential hypolipidemic activity. *International Journal of Pharmac and Bio Sciences,* 4(4), 729-740. Retrieved fromhttp://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.405.7204&rep=rep1&type=pdf

Korepanov, A., Korobeinikova, A., Shestakov, S., Garber, M., & Gongadze, G. (2012). Protein L5 is crucial for *in vivo* assembly of bacterial 50S ribosomal 50S ribosomal subunit central protuberance. *Nucleic Acids Research,* 40(18), 9153-9159. doi: 10.1093/nar/gks676

Krisko, A., Copic, T., Gabaldon, T., Lehner, B., & Supek, F. (2014). Inferring gene function from evolutionary change in signatures of translation efficiency. *Genome Biology,* 15(3), 1-17. doi: 10.1186/gb-2014-15-3-r44.

Kumar, V., Pandey, N., Mohan, V., & Singh, R. (2012). Antibacterial and antioxidant activity of extract of *Moringa oleifera* leaves-An in vitro study. *International Journal of Pharmaceutical Sciences Review and Research,* 12(1), 89-94. Retrieved from http://www.globalresearchonline.net/

LaPorte, D. (1993). The isocitrate dehydrogenase phosphorylation cycle: regulation and enzymology. *Journal of Cell Biochemistry,* 51(1), 14-18. doi: 10.1002/jcb.240510104

Lar, P., Ojile, E., Dashe, E., & Oluoma, J. (2011). Antibacterial activity of *Moringa oleifera* seed extracts on some gram-negative bacterial isolates. *African Journal of Natural Sciences,* 14, 57-62. Retrieved from http://www.ajol.info/index.php/index/browse/category?categoryId=16

Mahbub, K. R., Hoq, M., Ahmed, M. M., & Sarker, A. (2011). *In Vitro* antibacterial activity of *Crescentia cujete* and *Moringa oleifera*. *Bangladesh Research Publications Journal,* 5(4), 337-43. Retrieved from http://www.bdresearchpublications.com/admin/journal/upload/09236/09236.pdf

Mahmood, K., Mugal, T., & Haq, I. (2010). *Moringa oleifera*: A natural gift- A review. *Journal of Pharmaceutical Sciences and Research,* 2(11), 775-781. Retrieved from http://www.jpsr.pharmainfo.in/Documents/Volumes/Vol2Issue11/jpsr%2002101115.pdf

Makkar, H., & Becker, K. (1996). Nutritional value and antinutritional components of whole and ethanol extracted *Moringa oleifera* leaves. *Anim. Feed Sci. Tech.,* 63(1-4), 211-228. doi: http://dx.doi.org/10.1016/S0377-8401(96)01023-1

Mbikay, M. (2012). Therapeutic potential of *Moringa oleifera* leaves in chronic hyperglycemia and dyslipidemia: a review. *Frontiers in Pharmacology*, 3(24), 1-12. doi: 10.3389/fphar.2012.00024

McGuire, W. (2004). Infection in the preterm infant. *British Medical Journal,* 329(7477), 1277-1280. doi: 10.1136/bmj.329.7477.1277

Moyo, B., Masika, P. & Muchenje, V. (2012). Antimicrobial activities of *Moringa oleifera lam* extracts. *African Journal of Biotechnology,* 11(11), 2797-2802. Retrieved from http://www.academicjournals.org/AJB

Muhammad, A., Pauzi, N., Arulselvan, P., Abas, F., & Fakurazi, S. (2013). *In Vitro* wound healing potential and identification of bioactive compounds from *Moringa oleifera Lam. Biomed Research International,* 2013, 10 pages. Retrieved from http://dx.doi.org/10.1155/2013/974580

Oluduro, A. O. (2012). Evaluation of Antimicrobial properties and nutritional potentials of *Moringa oleifera Lam*. in South-Western Nigeria. *Malaysian Journal of Microbiology,* 8(2), 59-67. Retrieved from http://mjm.usm.my/uploads/issues/259/Research%201.pdf

Omojate, G., Enwa, F., Jewo, A., & Eze, C. (2014). Mechanisms of antimicrobial actions of phytochemicals against enteric pathogens – A Review. *Journal of Pharmaceutical, Chemical, and Biological Sciences,* 2(2), 77-85. Retrieved from http://www.jpcbs.info

Onyekaba, T., Omojate, C., & Anowi, F. (2013). Phytochemical screening and investigations of antibacterial activities of various fractions of the ethanol leaves extract of *Moringa oleifera lam* (*Moringaceae*). *Journal of Pharmaceutical, Chemistry, and Biological Sciences,* 3(3), 962-973. Retrieved from http://www.ijpcbs.com/files/70-3184.pdf

Ovalle, A., & Levancini, M. (2001). Urinary tract infections in pregnancy. *Current Opinion In Urology,* 11(1), 55-59. Retrieved from https://www.researchgate.net/profile/Alfredo_Ovalle/publication/12180145_Urinary_tract_infections_in_pregnancy/links/0fcfd50e30ea7e2dcc000000.pdf

Pal, S. K, Mukherjee, P., & Saha, B. (1995). Studies on the antiulcer activity of *Moringa oleifera* leaf extract on gastric ulcer models in rats. *Phytotherapy Research,* 9(6), 463-465. doi: 10.1002/ptr.2650090618

Prieto, A., Kahramanoglou, C., Ali, R., Fraser, G., Seshasayee, A., & Luscombe, N. (2011). Genomic analysis of DNA binding and gene regulation by homologous nucleoid-associated proteins IHF and HU in *Escherichia coli* K12. *Nucleic Acids Research,* 40(8), 3524-3537. doi: 10.1093/nar/gkr1236.

Rafailidis P., Ioannidou, E., & Falagas, M. (2007). Ampicillin/sulbactam: Current status in severe bacterial infections. *Drugs,* 67(13), 1829-1849. Retrieved from http://link.springer.com/journal/volumesAndIssues/40265

Rahman, M. M., Sheikh, M. M. I, Sharmin, S. A., Islam, M. S., Rahman, M. A., Rahman, M. M., & Alam, M. F. (2009). Antibacterial activity of leaf juice and extracts of *Moringa oleifera Lam.* against some

human pathogenic bacteria. *Chiang Mai University Journal of Natural Sci*ences, 8(2), 219-227. Retrieved from http://cmuj.cmu.ac.th/journalissue-cmujofnaturalsciences.php

Rao, A., Devi, P., & Kamath, R. (2001). *In vivo* radioprotective effect of *Moringa oleifera* leaves. *Indian Journal of Experimental Biology,* 39(9), 858-863. Retrieved from http://nopr.niscair.res.in/bitstream/123456789/24000/1/IJEB%2039(9)%20858-863.pdf

Reddy, U. M., Zhang, J., Sun, L., Chen, Z., Raju, T. N., & Laughon, S.K. (2012). Neonatal mortality by attempted route of delivery in preterm birth. *American Journal of Obstetrics and Gynecology,* 207(2), 1-14. doi:10.1016/j.ajog.2012.06.023.

Reed, J., Vo, T., Schilling, C. & Palsson, B. (2003). An expanded genome-scale model of *Escherichia coli* K-12 (iJR904 GSM/GPR). *Genome Biology,* 4(9), R54. doi: 10.1186/gb-2003-4-9-r54.

Romero, R., Avila, C. Santhanam, U. & Sehgal, P. B. (1990). Amniotic fluid interleukin 6 in preterm labor: association with infection. *Journal of Clinical Investigation,* 85(5), 1392-1400. doi: 10.1172/JCI114583

Romero, R., Espinoza, J., Goncalves, L. F., Kusanovic, J. P., Friel, L. A., & Nien, J. k.. (2006). Inflammation in preterm and term labor and delivery. *Seminars in Fetal Neonatal Medicine,* 11(5), 317-326. doi: 10.1016/j.siny.2006.05.001

Sharma, P., Jain, A., Pahwa, R., & Yar, M. (2010). Ciprofloxacin: Review on developments in synthetic, analytical, and medicinal aspects. *Journal of Enzyme Inhibition and Medicinal Chemistry,* 25(4), 577-589. doi: 10.3109/14756360903373350

Shimizu, K. (2013). Metabolic regulation of a bacterial cell system with emphasis on *Escherichia coli* metabolism. *International Research Scholarly Notices Biochemistry,* 2013, 1-47. Retrieved from http://www.hindawi.com/journals/isrn/2013/645983/

Siddhuraju, P., & Becker, K. (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree *(Moringa oleifera Lam.)* leaves. *Journal of Agricultural Food Chemistry,* 51(8), 2144-2155. doi: 10.1021/jf020444+

Spierer, P., & Zimmermann, R. (1978). Stoichiometry, cooperativity, and stability of interactions between 5S RNA and proteins L5, L18, and L25 from the 50S ribosomal subunit of *Escherichia coli. Biochemistry,* 17(13), 2474-2479. Retrieved from http://pubs.acs.org/journal/bichaw

Sreelatha, S., Jeyachitra, A., & Padma, P. (2011). Antiproliferation and induction of apoptosis by *Moringa oleifera* leaf extract on human cancer cells. *Food Chemistry Toxicology,* 49(6), 1270-1275. doi: 10.1016/j.fct.2011.03.006

Stohs, S., & Hartman, M. (2015). Review of the safety and efficacy of *Moringa oleifera*. *Phytotherapy Research,* 29(6), 796-804. doi: 10.1002/ptr.5325

Sutherland, P., & McAlister-Henn, L. (1985). Isolation and expression of the *Escherichia coli* gene encoding malate dehydrogenase. *Journal of Bacteriology,* 163(3), 1074-1079. Retrieved from http://jb.asm.org/content/163/3/1074.long

Takahashi-Iniguez, T., Aburto-Rodriguez, N., Vilchis-Gonzalez, A., & Flores, M. (2016). Function, kinetic properties, crystallization, and regulation of microbial malate dehydrogenase. *Journal of Zhejiang University Science B,* 17(4), 247-261. doi: 10.1631/jzus.B1500219

Tam, R., & Saier Jr., M. (1993). Structural, functional, and evolutionary relationships among extracellular solute-binding receptors of bacteria. *Microbiology Reviews,* 57(2), 320-346. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC372912/pdf/microrev00025-0036.pdf

Taylor, A., Hu, G., Hart, P., & McAlister-Henn, L. (2008). Allosteric motions in structures of yeast NAD+-specific isocitrate dehydrogenase. *Journal of Biological Chemistry,* 283(16), 10872-10880. doi: 10.1074/jbc.M708719200

Thilza, I., Sanni, S., Zakari, A., Sanni, F., Talle, M., & Joseph, M. (2010). *In vitro* antimicrobial activity of water extract of *Moringa oleifera* leaf stalk on bacteria normally implicated in eye diseases. *Science Publications,* 2(6), 80-82. Retrieved from http://www.sciencepub.net/academia/aa0206/13_2681_moringa_aa0206_80_82.pdf

Tiloke, C., Phulukdaree, A., & Chuturgoon, A. A. (2013). The antiproliferative effect of *Moringa oleifera* crude aqueous leaf extract on cancerous human alveolar epithelial cells. *BioMed Central Complementary & Alternative Medicine,* 13(226), 1-8. doi: 10.1186/1472-6882-13-226.

Verma, A., Vijayakumar, M., Mathela, C., & Rao, C. (2009). *In vitro* and *in vivo* antioxidant properties of different fractions of *Moringa oleifera* leaves. *Food Chem. Toxicol.,* 47(9), 2196-2201. doi: 10.1016/j.fct.2009.06.005

Zakeri, B., & Wright, G. (2008). Chemical biology of tetracycline antibiotics. *Biochemistry and Cell Biology,* 86(2), 124-136. doi: 10.1139/O08-002

# Tables

**Table 1: Different measurements used in preparing *Moringa oleifera* stock extracts.**

| Label | Amounts of Solution | Total (µL) | Concentration (mg/mL) |
|---|---|---|---|
| **$H_2O$** | 290 µL Extract + 710 µL PBS | 1000 | 1 |
| **Ethanol/$H_2O$ (80/20)** | 322 µL Extract + 678 µL PBS | 1000 | 1 |
| **Ethanol** | 357 µL Extract + 633 µL PBS | 1000 | 1 |
| **Methanol** | 48 µL Extract + 952 µL PBS | 1000 | 1 |
| **Butanol** | 625 µL Extract + 375 µL PBS | 1000 | 1 |

**Table 2: Parameters used for proteomics analysis in PEAKS program.**

| **Search Engine** | **PEAKS** |
|---|---|
| **Percent Mass Error Tolerance** | **50.0 ppm** |
| **Fragment Mass Error Tolerance** | **0.5 Da** |
| **Precursor Mass Search Type** | **monoisotopic** |
| **Enzyme** | **Trypsin** |
| **Max Missed Cleavages** | **3** |
| **Non-specific Cleavage** | **both** |
| **Carbamidomethylation (C)** | **57.02** |
| **Oxidation (M)** | **15.99** |
| **Formylation (Protein N-term)** | **27.99** |
| **Max variable PTM per peptide** | **3** |
| **Database** | **Uniprot_Sprot** |
| **Taxon** | ***Escherichia coli*** |

**Table 3. Represents the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of five different *M. oleifera* extracts on *E. coli* growth** (n=3).

| Extraction Solvent | MIC (µg/mL) | MBC (µg/mL) |
|---|---|---|
| **Ethanol** | 0.75 | 3 |
| **Ethanol/Water (80/20)** | 1.5 | 7-8 |
| **Butanol** | >20 | >20 |
| **Methanol** | >20 | >20 |
| **Water** | >20 | >20 |

***Table 4. Comparison of inhibition of E. coli by the antibiotic ciprofloxacin (5µg) to the ethanolic M. oleifera whole leaf extract at various concentrations.*** Ethanolic *M. oleifera* whole leaf extract (20µg) is able to produce the same inhibition zone as ciprofloxacin (5µg) against *E. coli,* but the extract at 25µg is able to inhibit 120% that of ciprofloxacin (5µg).

| Amount Substance(µg) | Ciprofloxacin Inhibition (mm) | Ethanolic Extract Inhibition (mm) | Antibiotic vs. Extract (%) |
|---|---|---|---|
| **5** | 28 | 17 | 61 |
| **10** | - | 21.5 | 77 |
| **15** | - | 25.5 | 91 |
| **20** | - | 28 | 100 |
| **25** | - | 33.5 | 120 |

**Table 5.** ***LD50 results for ethanolic M. oleifera extract and common antibiotics when treatment was able to reduce bacteria by half of the controls group absorbance.*** Data shows that the Ethanolic *M. oleifera* extract requires the least amount of extract to inhibit 50% of *E. coli* growth (n=3).

| | **$LD_{50}$ (µg/mL)** |
|---|---|
| Ethanolic *Moringa oleifera* extract | 2-2.5 |
| Tetracycline | 7.5 |
| Streptomycin | 4-5 |
| Ampicillin | 12.5 |

**Table 6. Proteins identified as significantly changed during proteomics analysis.**

| Protein Name | Gene | Process Involvement | Expression Change | Enhanced/ Down-regulated |
|---|---|---|---|---|
| Oxidoreductase YajO | yajO | Metabolism | 1.91 | Enhanced |
| Malate dehydrogenase (MDH) | mdh | Metabolism | 2.13 | Enhanced |
| Ribosomal large subunit pseudouridine synthase B | rluA | Ribsome Synthesis | 1.95 | Enhanced |
| ATP synthase subunit c | atpE | ATP Synthesis | 1.87 | Enhanced |
| Isocitrate dehydrogenase [NADP] (IDH) | icd | Stress Response | 2.08 | Enhanced |
| Succinyl-CoA ligase [ADP-forming] subunit beta (SUCC) | sucC | ATP Synthesis | 1.98 | Enhanced |
| DNA-binding protein HU-alpha | hupA | Stress Response | 0.71 | Down-regulated |
| Succinyl-CoA ligase [ADP-forming] subunit alpha (SUCD) | sucD | ATP Synthesis | 2.29 | Enhanced |
| 50S ribosomal protein L5 | rplE | Ribsome Synthesis | 0.65 | Down-regulated |
| Ornithine carbamoyltransferase chain F | argI | Stress Response | 6.64 | Enhanced |
| Glutamate/aspartate periplasmic-binding protein | ybeJ | Periplasmic-binding | 2.84 | Enhanced |
| Enoyl-[acyl-carrier-protein] reductase [NADH] FabI | fabI | Membrane Transport | 2.11 | Enhanced |
| Alcohol dehydrogenase propanol-preferring (ADHP) | adhP | Stress Response | 3.19 | Enhanced |

## Figures

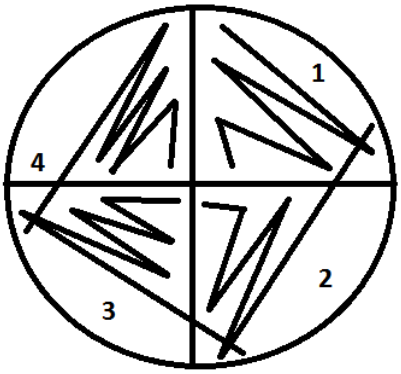



***Figure 1: Demonstrates streaking technique used to grow bacteria.***

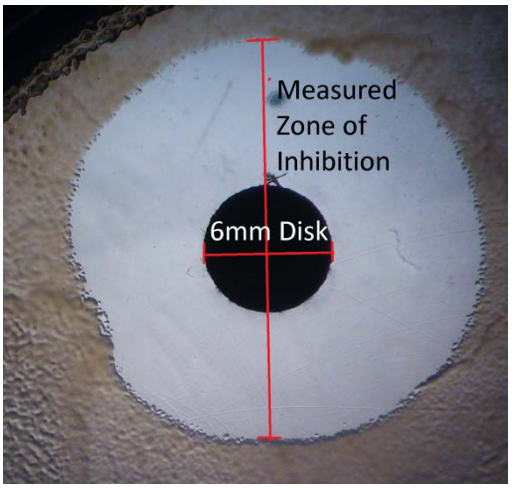



***Figure 2. Measurement of the zone of Inhibitions:*** This Figure shows a diffusion assay of the *M. oleifera* ethanol extract at 10µg with the zone of inhibition and the 6 mm disk located in the center of the inhibition diameter. The tan area shows the *E. coli*, while the white area is the bacteria-free zone due to inhibition by ethanolic *Moringa* whole leaf extract and the black area is the 6 mm disk.

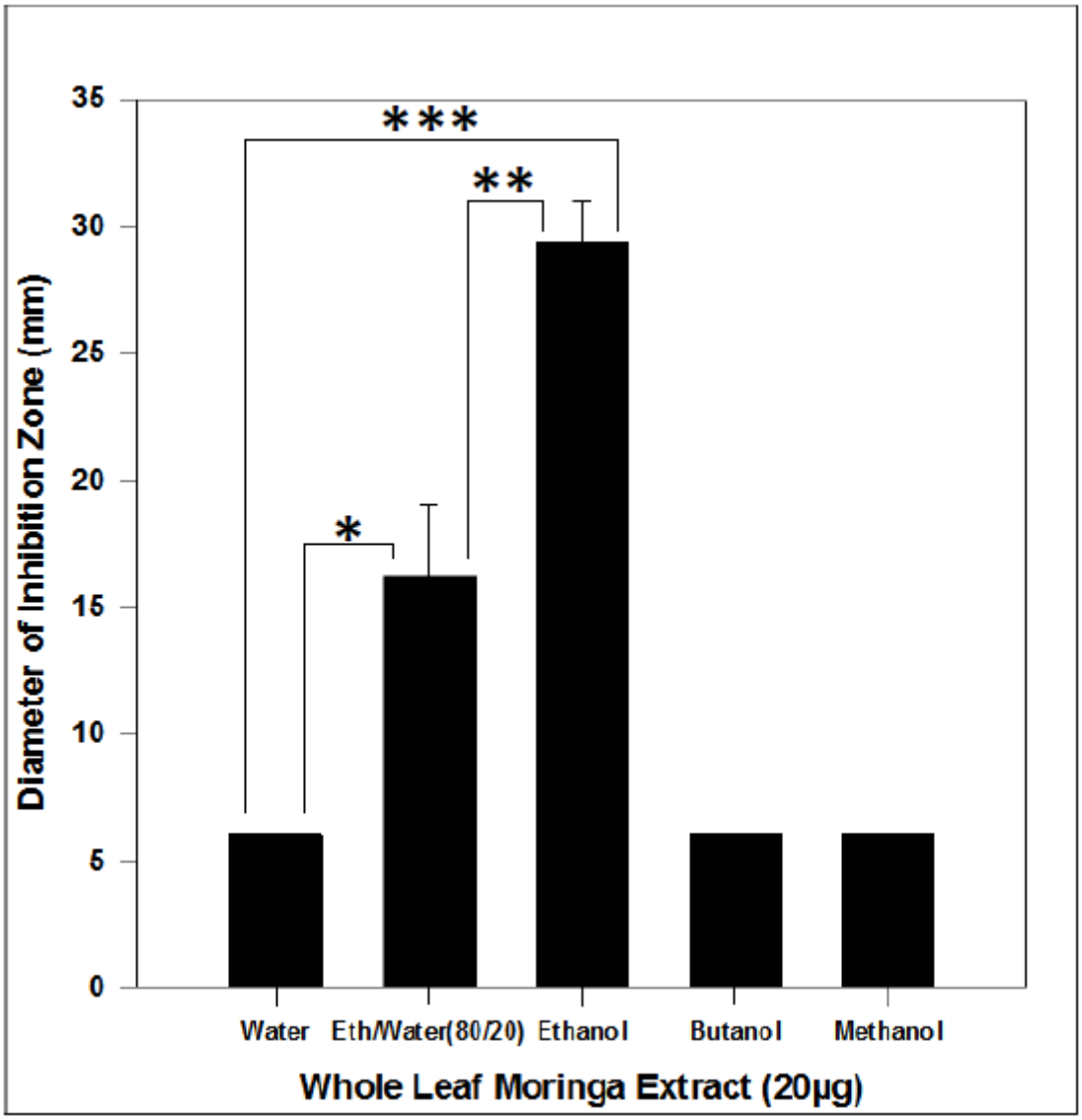



***Figure 3. Diffusion assay showing inhibition zones of five M. oleifera extracts at 20 µg against E. coli:*** Data shows significant bacterial growth inhibition by *M. oleifera* pure ethanol extract and ethanol/water (80/20) extract. Water, butanol and methanol extracts did not inhibit *E. coli* growth. Diameter of the discs used were 6mm and an inhibition of 6mm indicates no inhibition by the extract. * p ≤0.05 Eth/water vs. water; ** p ≤0.05 Ethanol vs. Eth/water; *** p≤0.05 Ethanol vs. water (n=3).

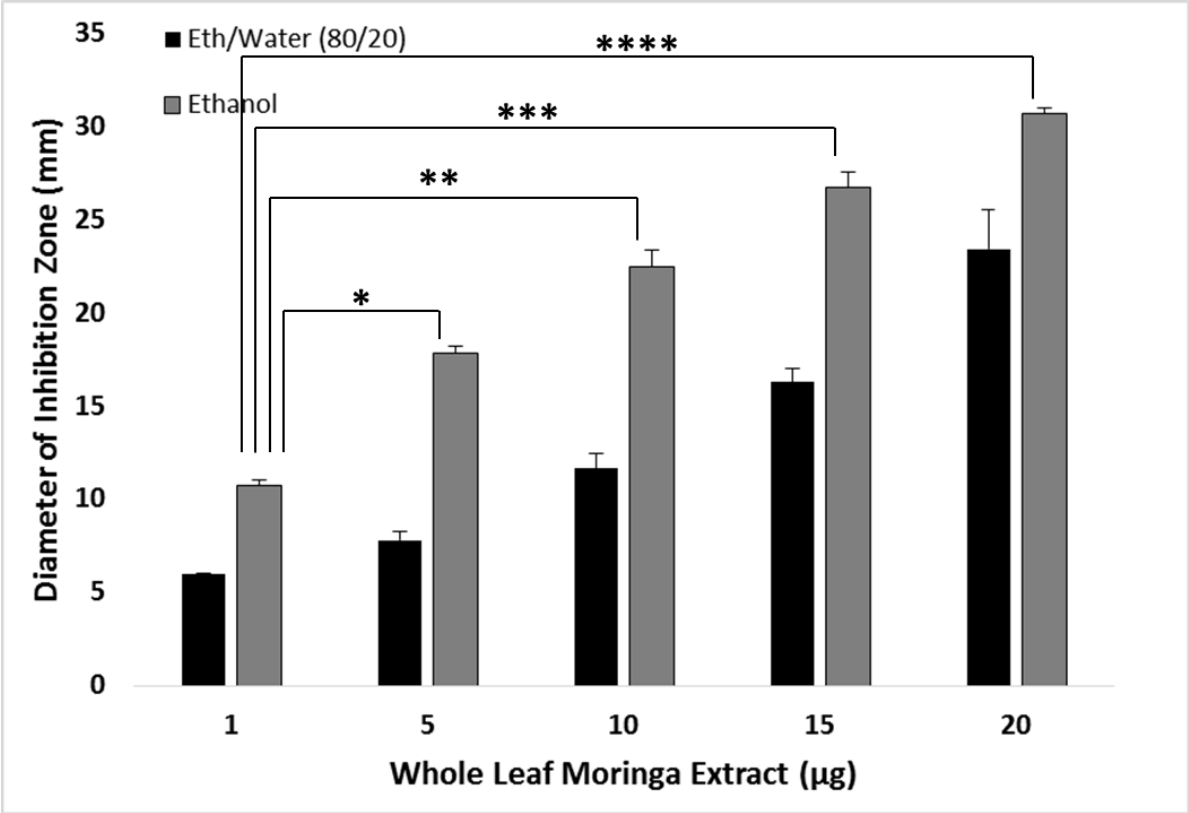



**Figure 4.** ***Diffusion assay showing diameter of the inhibition zone of M. oleifera pure ethanolic and hydro-ethanolic M. oleifera extracts at increasing amounts against E. coli:*** Data shows dose-dependent inhibition of bacterial growth by Diffusion assay for *M. oleifera* pure ethanolic and hydro-ethanolic Moringa extracts. The pure ethanolic extract displayed a more potent inhibitory effect on *E. coli* growth compared to the hydro-ethanolic extract. Diameter of the discs used were 6mm and an inhibition zone of 6mm indicates no inhibition by the extract. * $p \leq 0.05$, 1 vs. 5 µg; ** $p \leq 0.05$, 1 vs. 10 µg; *** $p \leq 0.05$, 1 vs. 15 µg; **** $p \leq 0.05$, 1 vs. 20 µg (n=3).

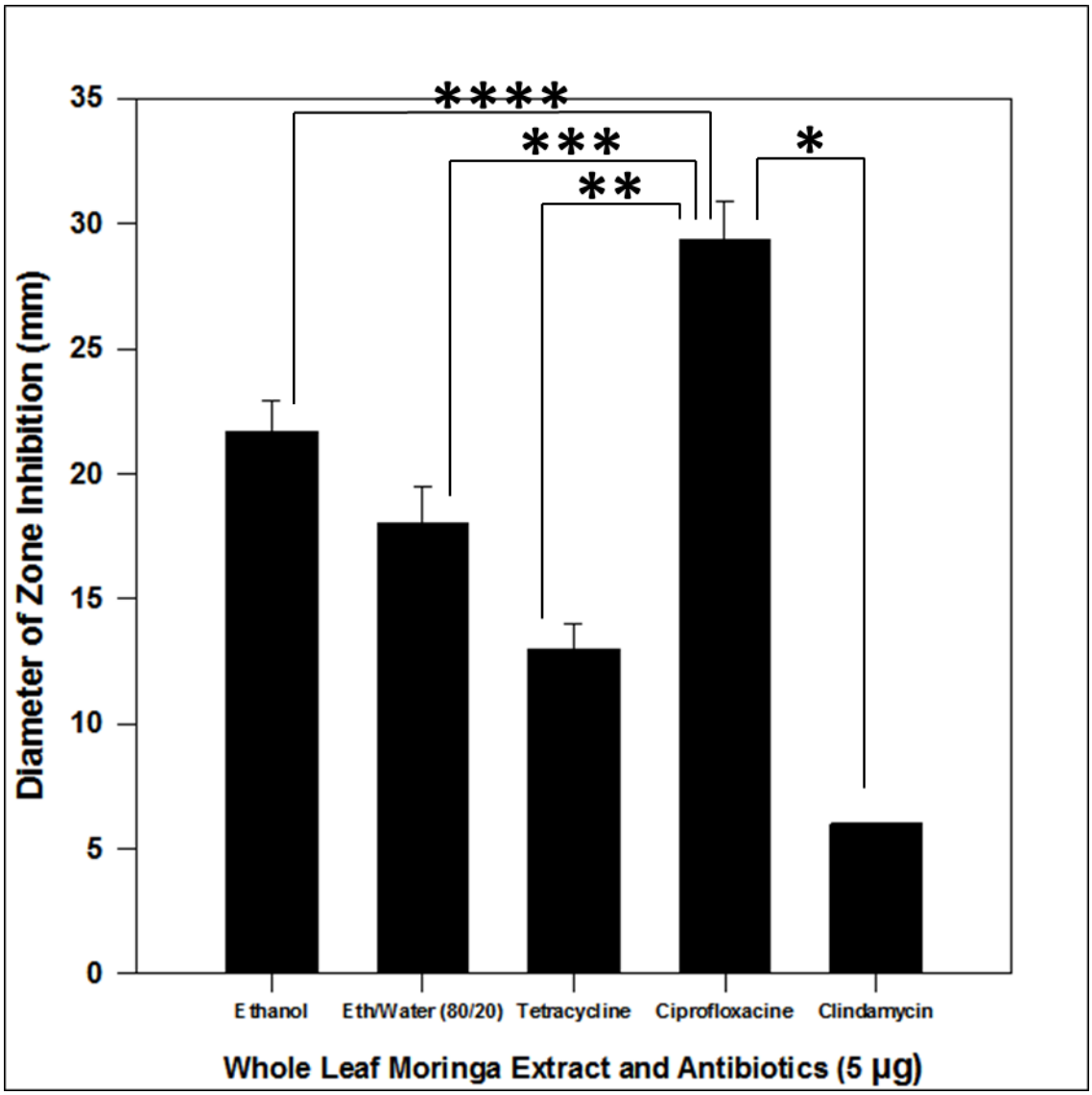
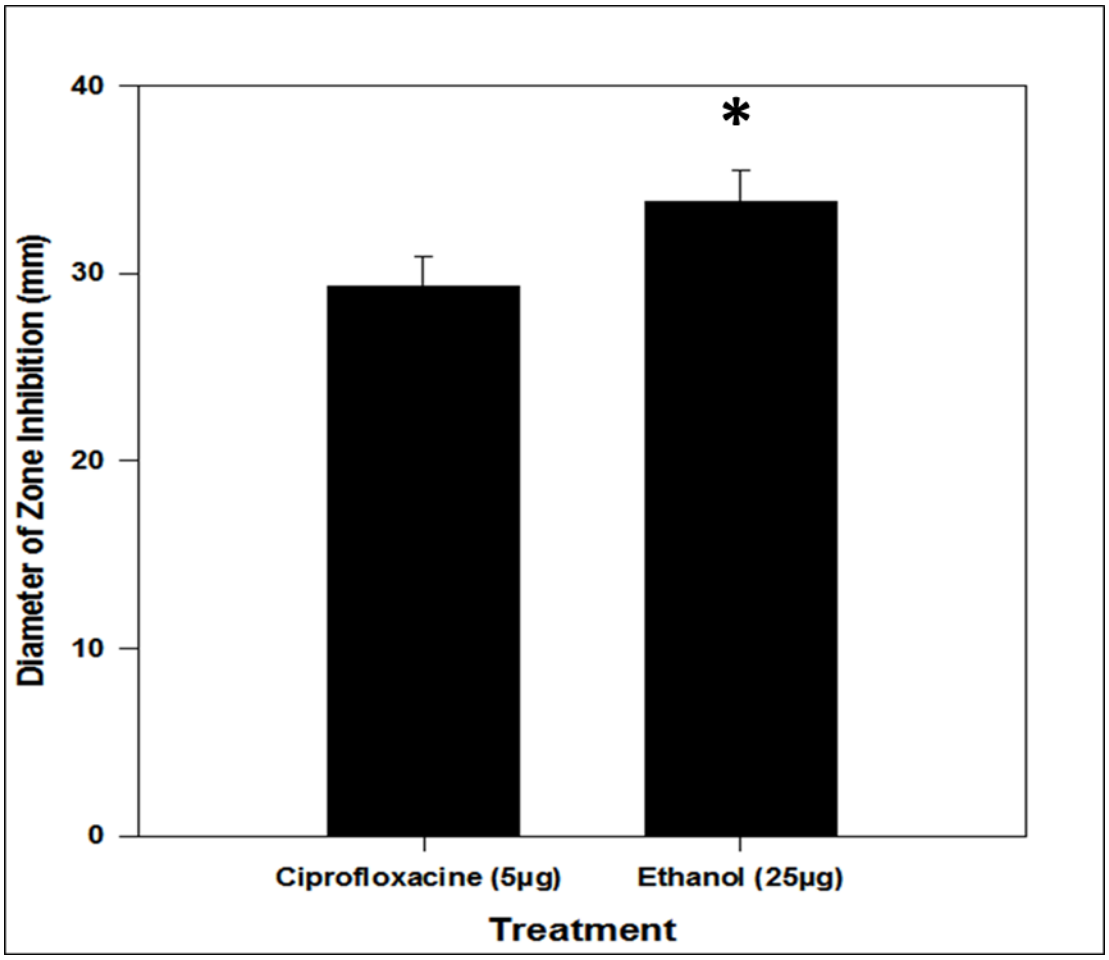



**Figure 5.** ***Comparisons of inhibition zones of M. oleifera pure and hydroethanolic M. oleifera extracts versus antibiotics (Tetracycline, ciprofloxacin, and clindamycin) at similar amounts (5 µg) against E. coli:*** The zone of inhibition of both *M. oleifera* ethanolic and hydro-ethanolic extracts were approximately two-thirds the size of ciprofloxacin but significantly larger than both tetracycline and clindamycin. Diameter of the discs used were 6mm and an inhibition zone of 6mm indicates no inhibition by the extract. *p≤0.01, Clindamycin vs. Ciprofloxacin; p ≤0.01, Tetracycline vs. Ciprofloxacine; *** p ≤0.05, Eth/Water vs. Ciprofloxacine; **** p ≤ 0.05, Ethanol vs. Ciprofloxacine (n=3).




**Figures 6.** ***Diffusion assay showing the respective amounts at which ciprofloxacin (5 µg) and pure ethanol M. oleifera extracts (25 µg) attain comparable diameter inhibition zones against E. coli:*** *M. oleifera* pure ethanolic whole leaf extract at 20/25 µg attains a greater diameter of zone of (*E. coli* growth) inhibition to ciprofloxacin at 5 µg. Diameter of the discs used were 6mm and an inhibition zone of 6mm indicates no inhibition by the extract. *p≤0.05, Ethanol (25µg) vs. Ciprofloxacin (5µg).

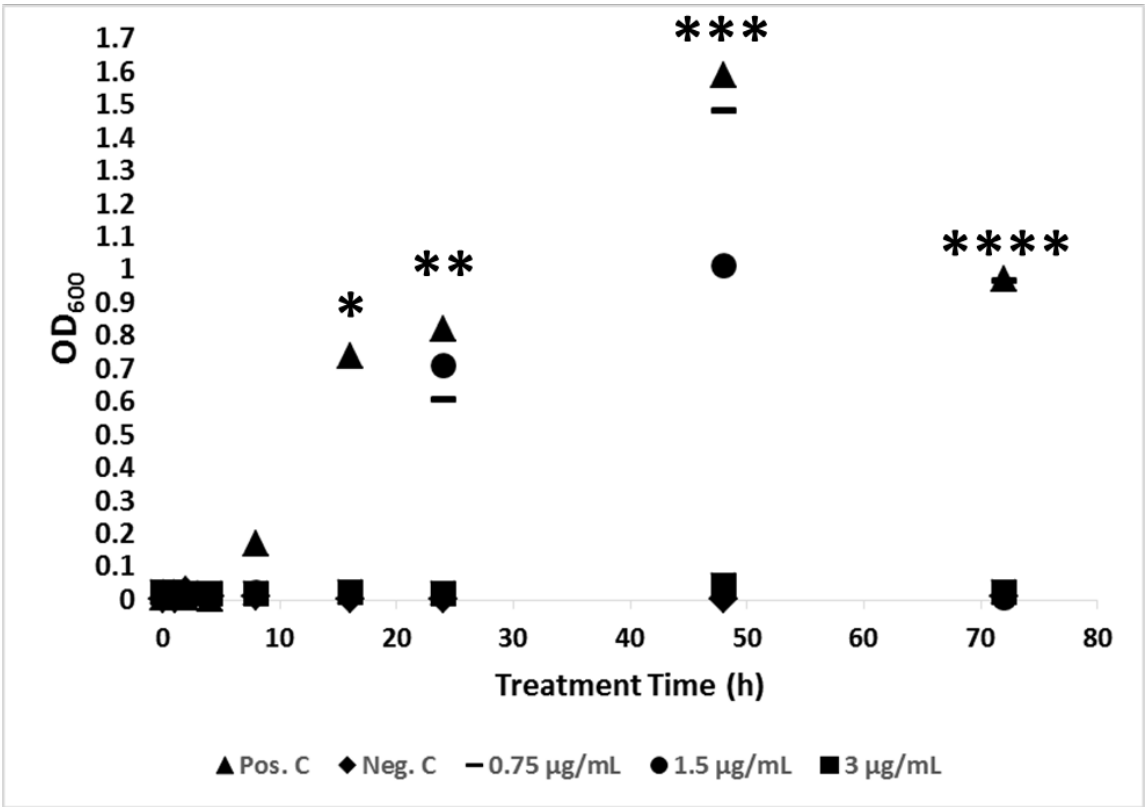



***Figure 7. Antibacterial kinetic plots of M. oleifera ethanol extract against 1500 CFU of E. coli cells:*** *M. oleifera* treatments show a longer lag phase and reduced growth for 3 µg treatment compared to positive control. *M. oleifera* treatment of 6 µg inhibited all growth of *E. coli*. * p ≤0.05, 3.0 µg/mL vs. Pos. C at 16h; ** p ≤ 0.05, 3.0 µg/mL vs. Pos. C. and 3.0 µg/mL vs. 0.75 µg/mL and 3.0 µg/mL vs. 1.5 µg/mL at 24h; *** p ≤ 0.05, 3.0 µg/mL vs. Pos. C. and 3.0 µg/mL vs. 0.75 µg/mL and 3.0 µg/mL vs. 1.5 µg/mL at 48h; **** p ≤ 0.05, 3.0 µg/mL vs. Pos. C. and 3.0 µg/mL vs. 0.75 µg/mL and 3.0 µg/mL vs. 1.5 µg/mL at 72h (n=3).

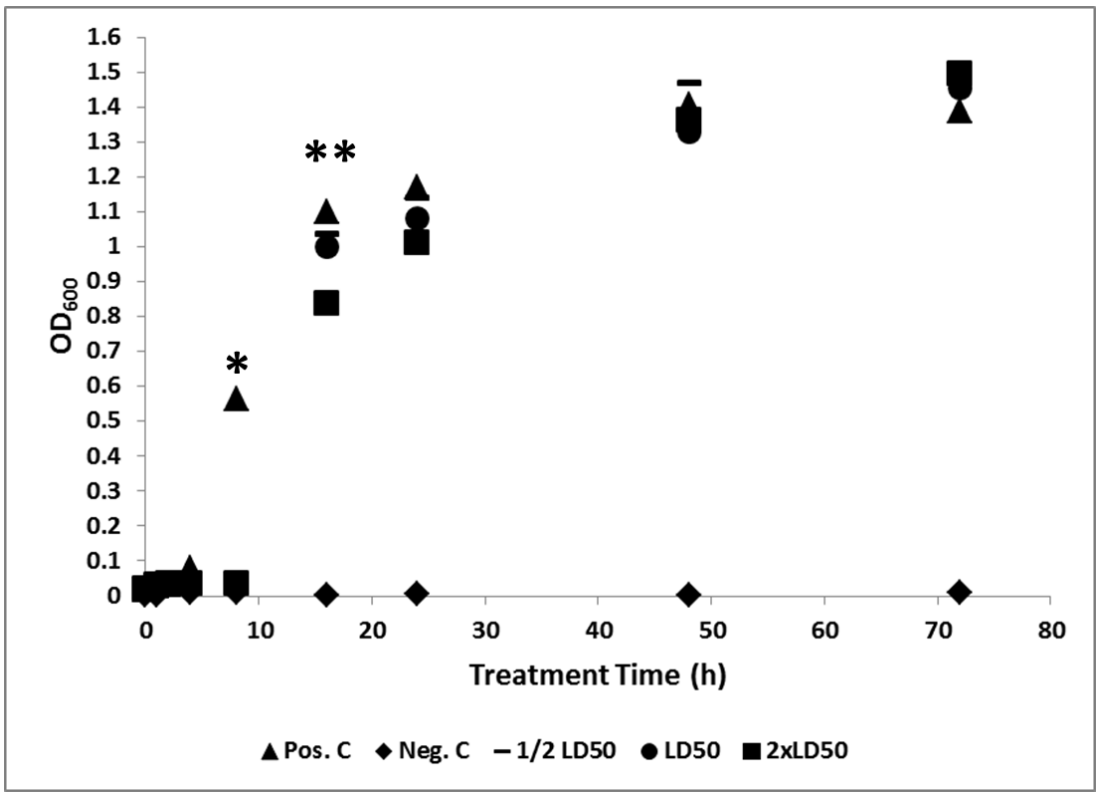



***Figure 8. Antibacterial kinetic plots of the antibiotic ampicillin against 1500 CFU of E. coli cells:*** Ampicillin treatments showed significant inhibition of *E. coli* cells at ½ LD50, LD50 and 2x LD50 at the 8h time point. The 2x LD50 ampicillin treatment showed significant inhibition of growth of *E. coli* cells at the 16h time point. *$p \leq 0.05$, Pos. C vs. ½ LD50 and Pos. C vs. LD50 and Pos. C vs. 2x LD50 at 8h; **$p \leq 0.05$, Pos. C vs. 2x LD50 at 16h (n=3).

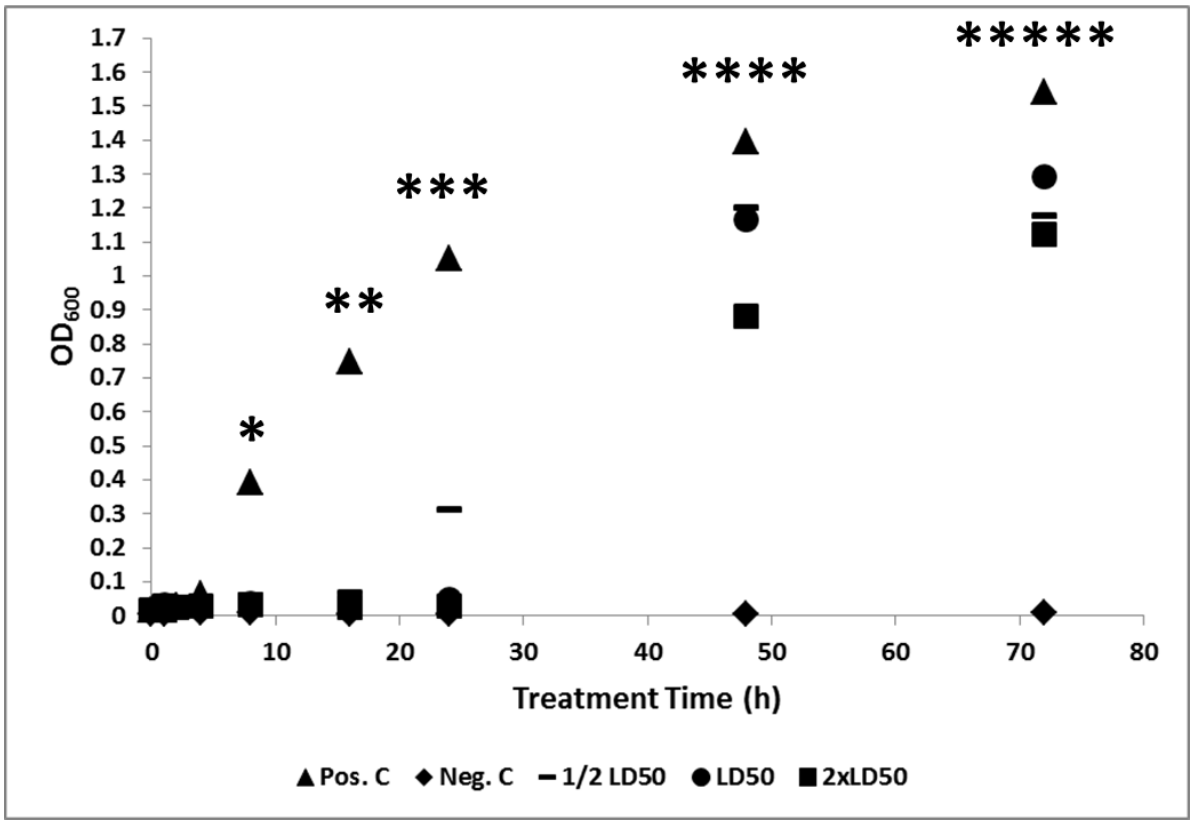



***Figure 9. Antibacterial kinetic plots of the antibiotic streptomycin against 1500 CFU of E. coli cells:*** Streptomycin treatments show a longer lag phase and reduced growth at 2xLD50 compared to the positive control. *p ≤ 0.05, Pos. C vs. LD50 and Pos. C vs. ½ LD50 and Pos. C vs. 2xLD50 at 8h; **p ≤ 0.05, Pos. C vs. ½ LD50 and Pos. C vs. LD50 and Pos. C vs. 2xLD50 at 16h; ***p ≤ 0.05, Pos. C vs. ½ LD50 and Pos. C vs. LD50 and Pos. C vs. 2xLD50 at 24h; ****p ≤ 0.05, Pos. C vs. 2xLD50 at 48h; *****p ≤ 0.05, Pos. C vs. 2xLD50 at 72h (n=3).

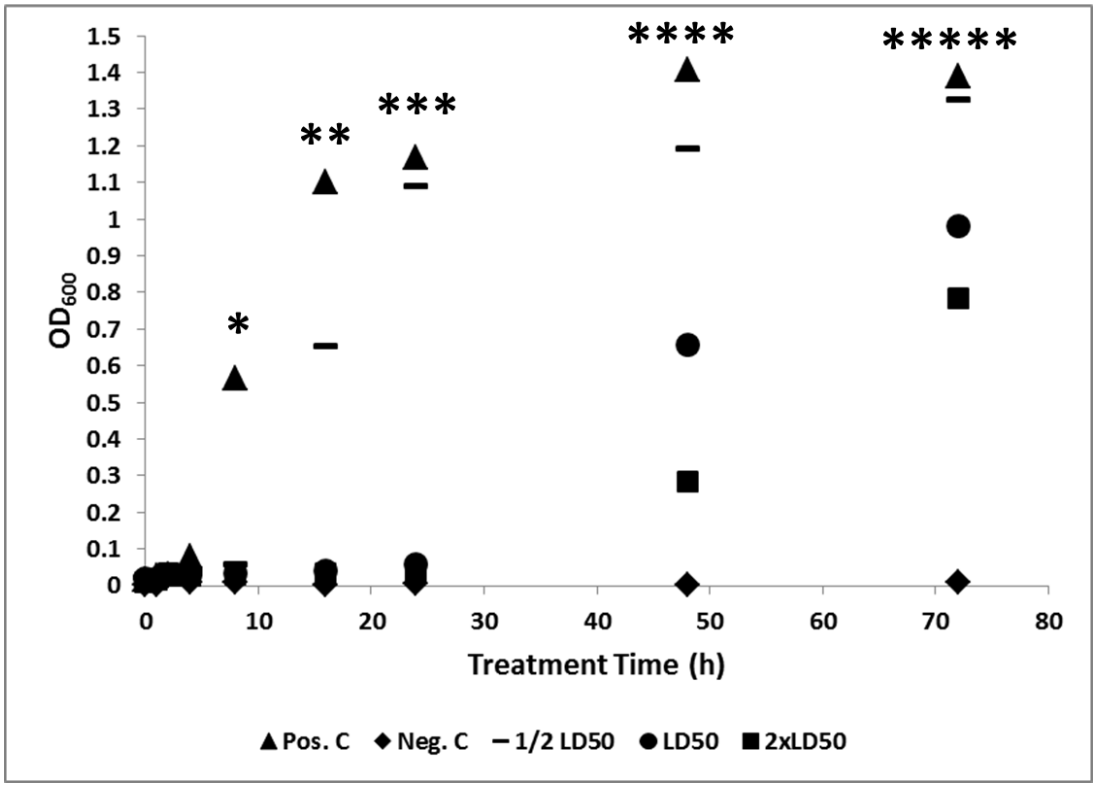



***Figure 10. Antibacterial kinetic plots of the antibiotic tetracycline against 1500 CFU of E. coli cells:*** Tetracycline treatments showed a longer lag phase and reduced growth at treatment concentrations of LD50 and 2x LD50 compared to the positive control. At a tetracycline treatment concentration of ½ LD50 a notable difference was observed at 8h, and 16h when compared to the positive control. *$p \leq 0.05$, Pos. C vs. ½ LD50 and Pos. C vs. LD50 and Pos. C vs. 2x LD50 at 8h; **$p \leq 0.05$, Pos. C vs. ½ LD50 and Pos. C vs. LD50 and Pos. C vs. 2x LD50 at 16h; ***$p \leq 0.05$, Pos. C vs. LD50 and Pos. C vs. 2x LD50 at 24h; ****$p \leq 0.05$, Pos. C vs. LD50 and Pos. C vs. 2x LD50 at 48h; *****$p \leq 0.05$, Pos. C vs. LD50 and Pos. C vs. 2x LD50 at 72h (n=3).

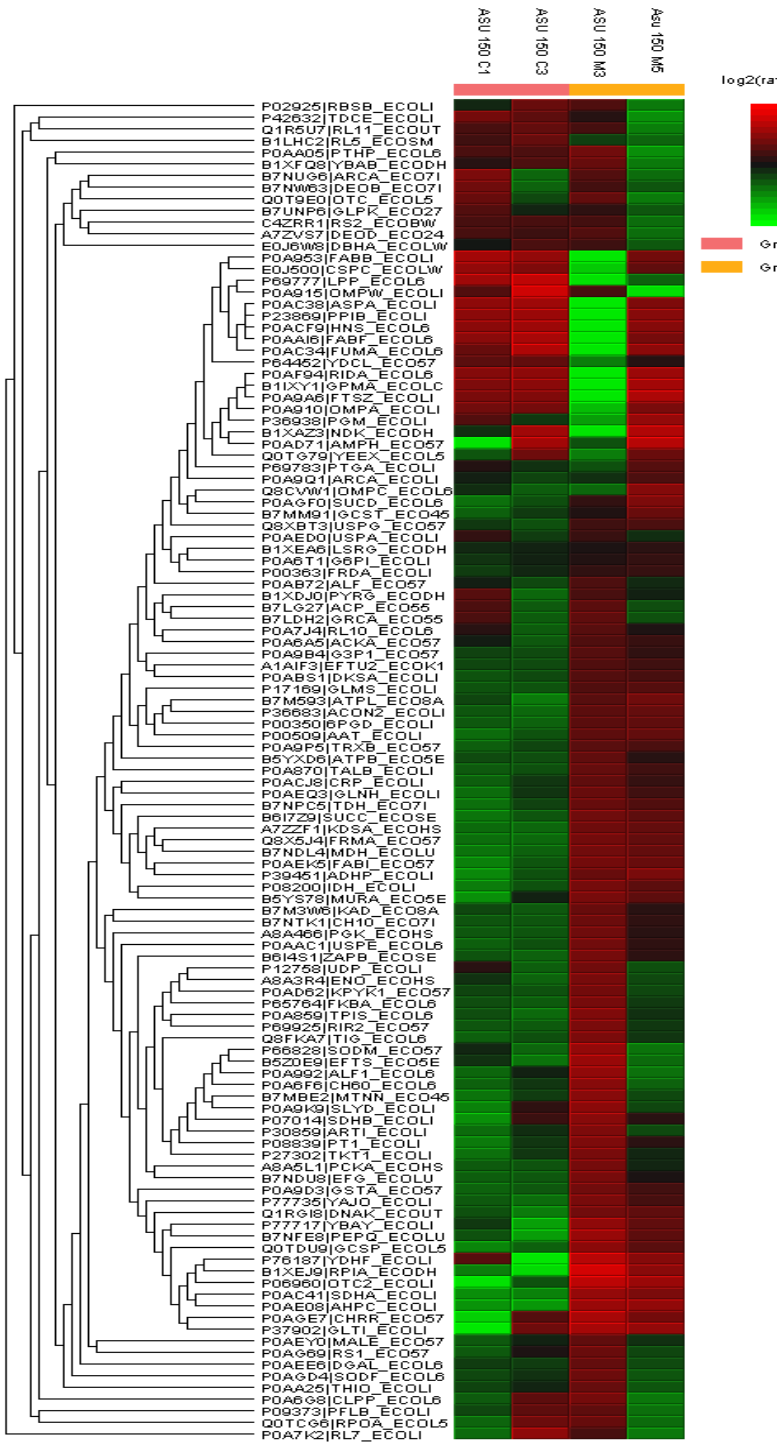



***Figure 11. Quantitative proteomics analysis of protein expression in E. coli treated with ethanolic whole leaf M. oleifera extract (2.5µg/mL).*** Approximately 64 proteins were identified, with a small subset demonstrating statistically significant changes in *E. coli* treated with *M. oleifera*. Red indicates higher protein expression compared to baseline control, and green indicates lower protein expression compared to baseline control. Control treatments (C1, C3), *M. oleifera* treatments (M3, M5).

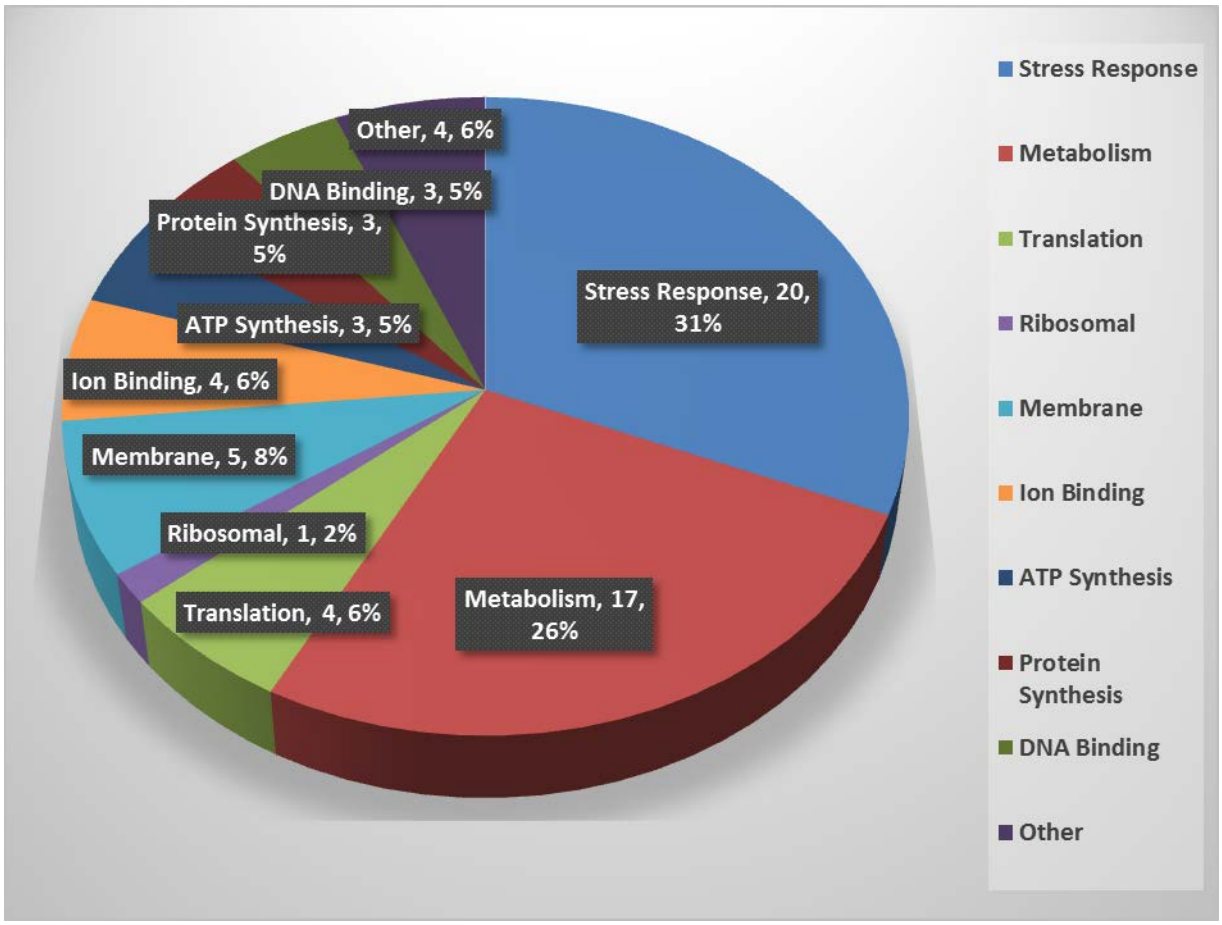



***Figure 12. M. oleifera modulates a plethora of key biological processes and protein expression in E. coli, such as ATP and protein synthesis, as well as factors involved in metabolism, stress response and membrane function, as revealed by proteomics analysis:*** Biological processes that are altered by protein expression when *E. coli's* treated with ethanolic *M. oleifera* whole leaf extract. Almost two thirds of the proteins are involved in metabolism and stress response. Of the 64 proteins changed during the ethanolic *M. oleifera* treatment, only 13 were significantly changed.

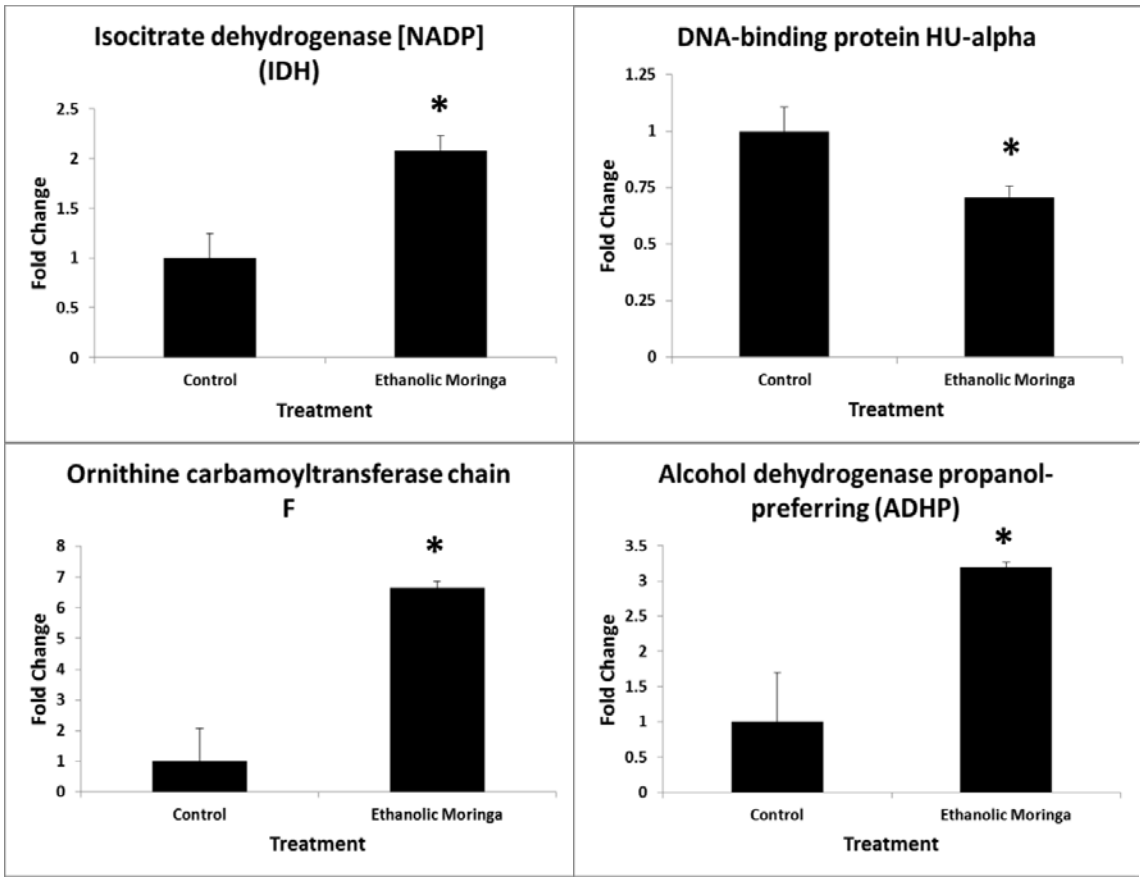



***Figure 13. Specific stress response proteins in E. coli altered by ethanolic extract of M. oleifera, as revealed by proteomics analysis.*** Four stress response proteins, namely isocitrate dehydrogenase (IDH), DNA-binding protein HU-alpha, ornithine carbamoyltransferase chain F and alcohol dehydrogenase propanol-preferring (ADHP) were variably expressed in *E. coli* treated with *M. oleifera* compared to the control group. Overall, these proteins were all significantly up regulated, except for DNA-binding protein HU-alpha, which was down regulated. *$p \leq 0.05$ (n=3).

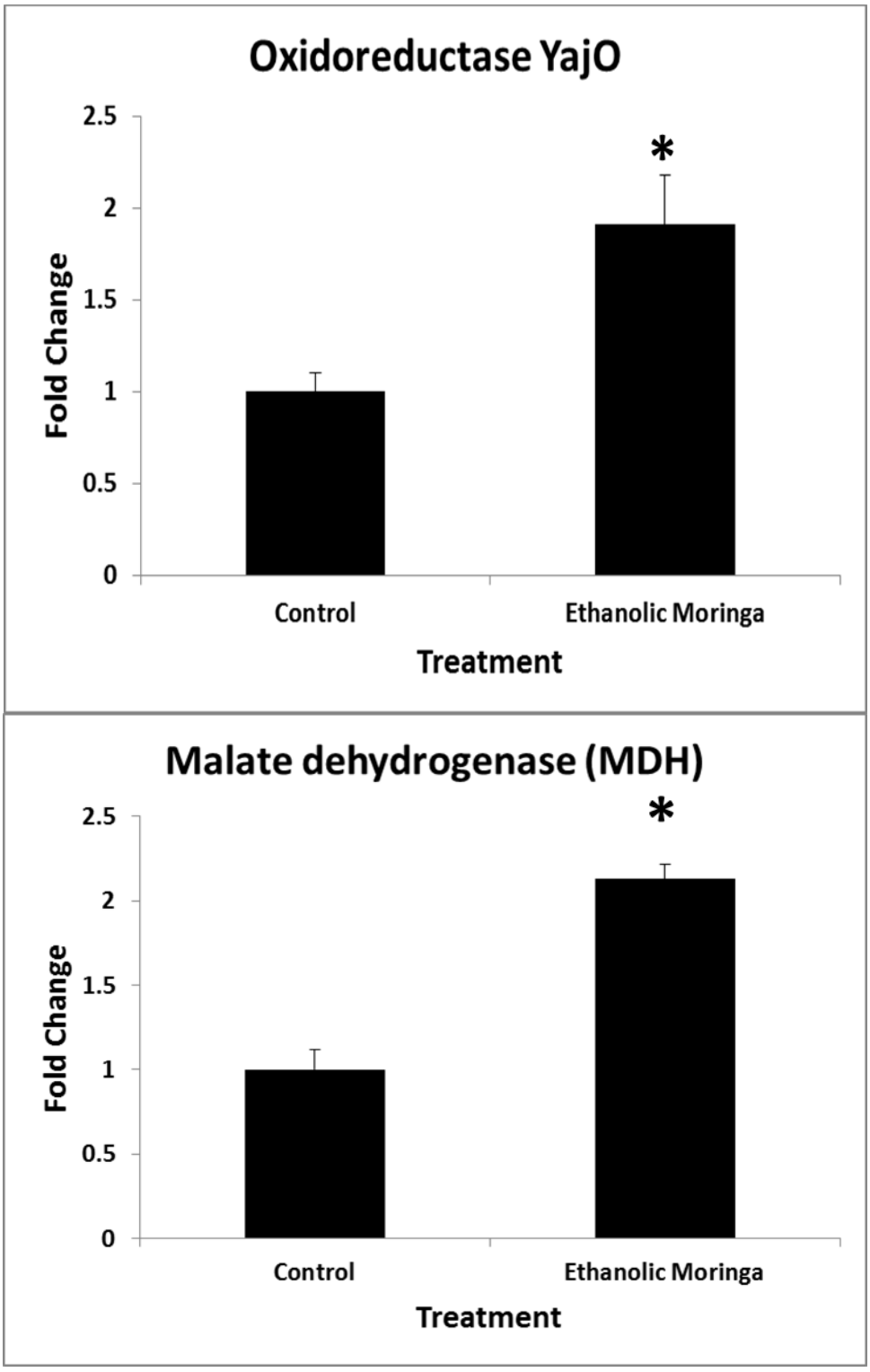



***Figure 14. Specific metabolic proteins in E. coli altered by ethanolic extract of M. oleifera, as revealed by proteomics analysis.*** Two metabolic proteins, namely Oxidoreductase YajO, and malate dehydrogenase (MDH) were variably expressed in *E. coli* treated with *M. oleifera* compared to the control group. These proteins were significantly up regulated. *p≤ 0.05 (n=3).

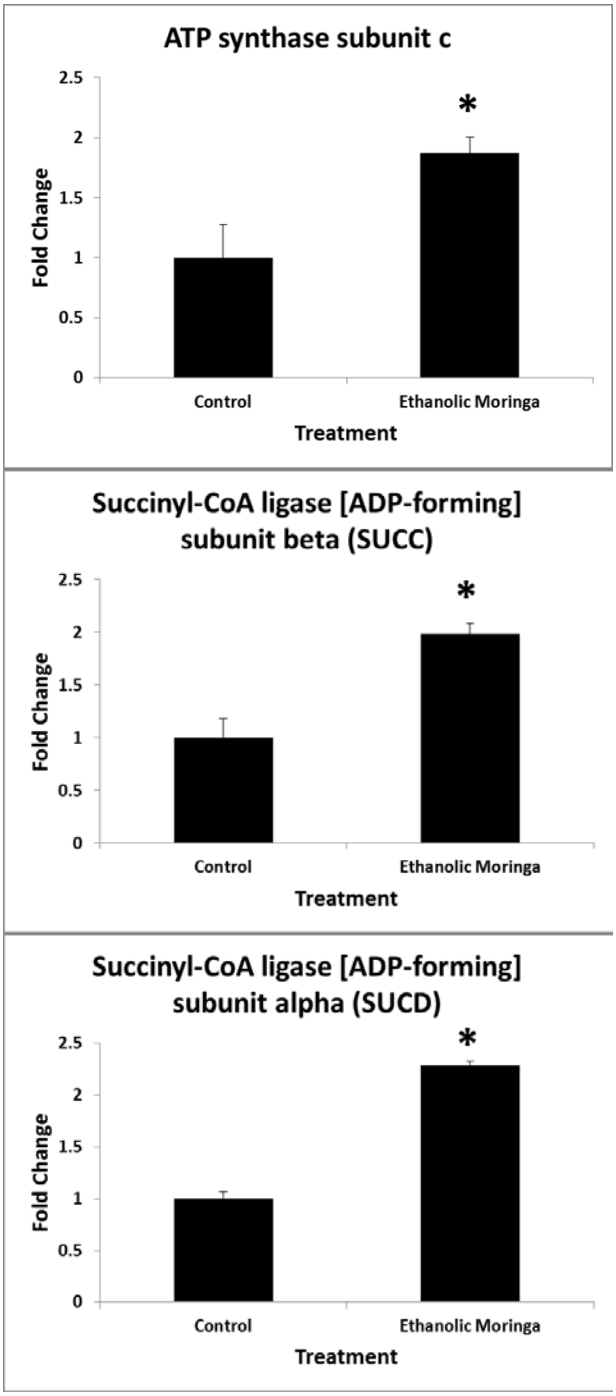



***Figure 15. Specific ATP synthesizing proteins in E. coli altered by ethanolic extract of M. oleifera, as revealed by proteomics analysis.*** Three ATP synthesizing proteins, namely ATP synthase subunit C, Succinyl-CoA ligase subunit beta (SUCC), and Succinyl-CoA ligase subunit alpha (SUCD) were variably expressed in *E. coli* treated with *M. oleifera* compared to the control group. These proteins were all significantly up regulated. *$p \leq 0.05$ (n=3).

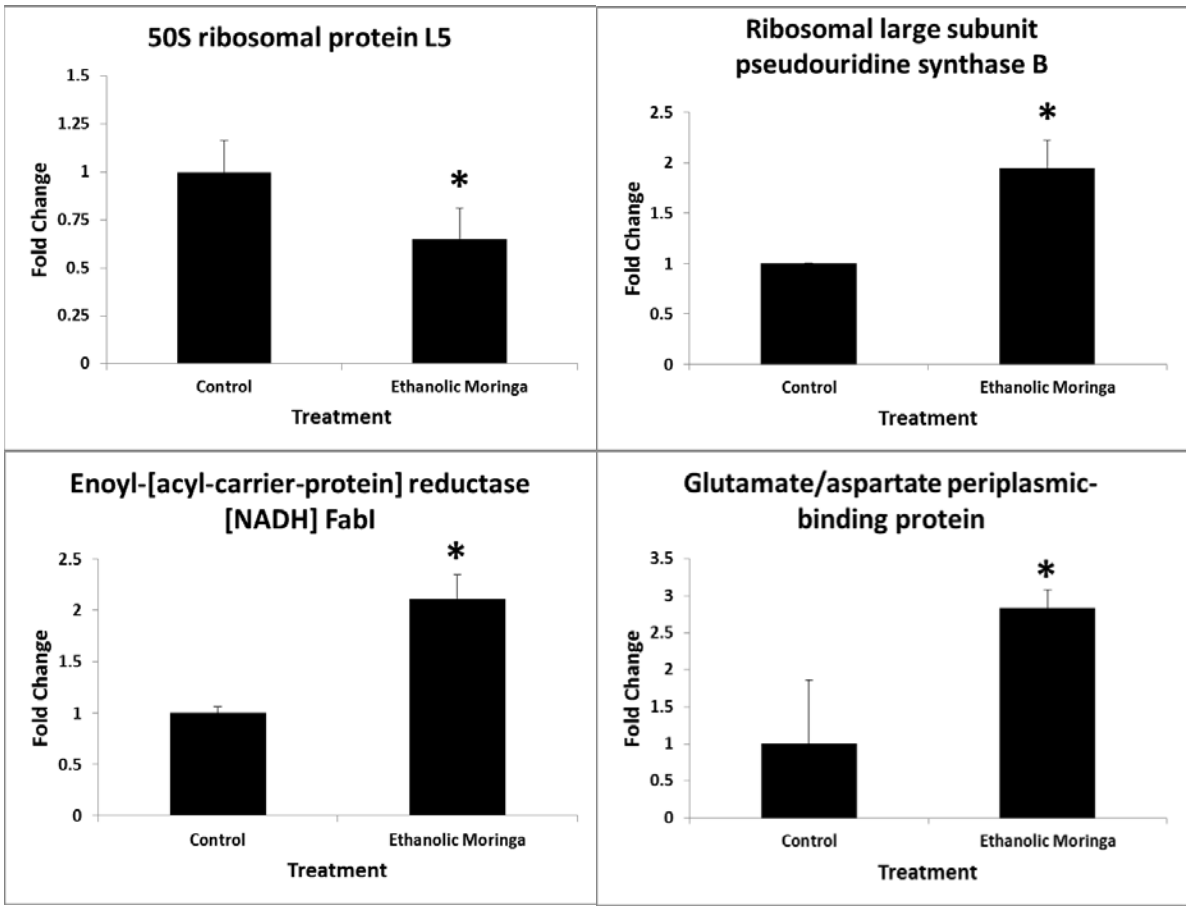



***Figure 16. A variety of proteins involved in various biological processes in E. coli altered by ethanolic extract of M. oleifera, including translation, ribosome synthesis, membrane synthesis and periplasmic-binding, as revealed by proteomics analysis.*** Various proteins involved in a cross section of biological functions in *E. coli*, including translation (50S ribosomal protein L5), ribosomal function (ribosomal large subunit pseudouridine synthase B), membrane function (Enoyl-[acyl-carrier-protein] reductase [NADH] FabI), and unknown (glutamate/aspartate periplasmic-binding protein, were variably expressed in *E. coli* treated with *M. oleifera* compared to the control group. Overall, all the proteins were significantly up regulated, except for 50S ribosomal protein 1.5, which was down regulated. *$p \leq 0.05$ (n=3).

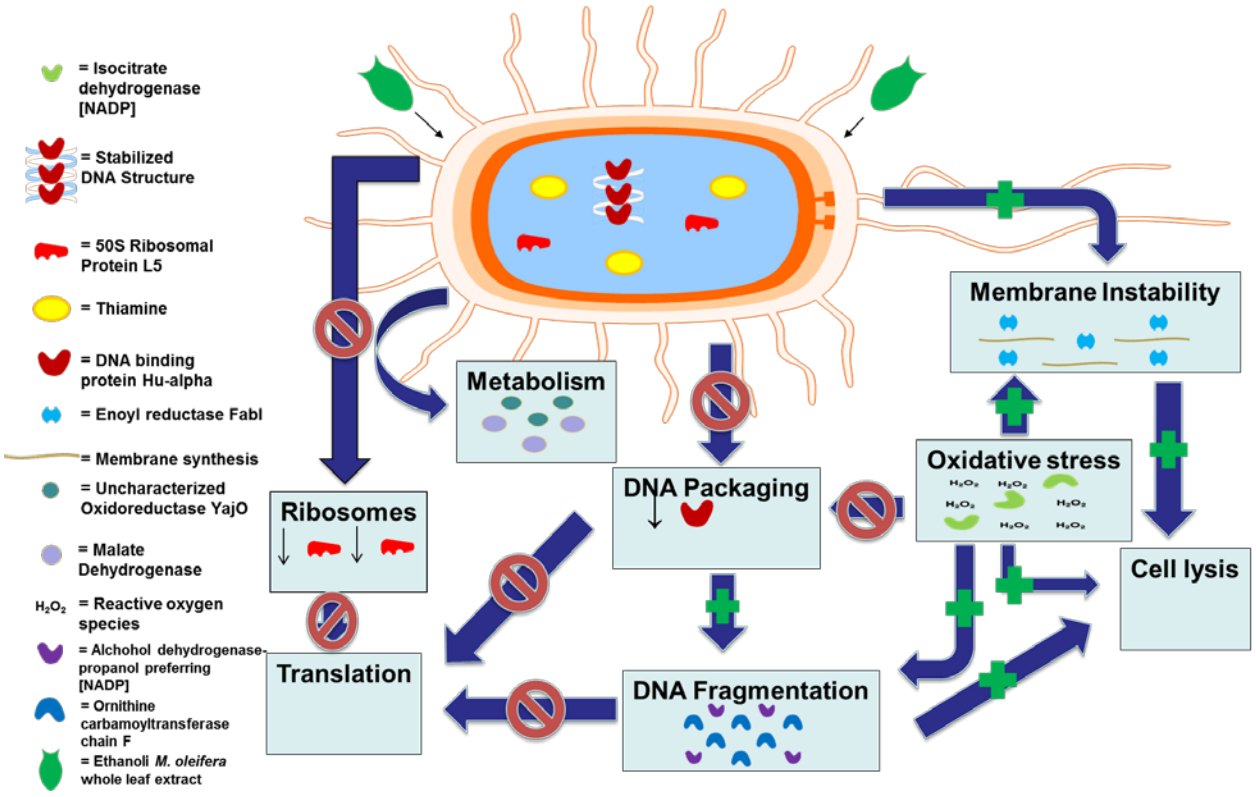



***Figure 17. Proposed mechanism of action of Moringa oleifera on Escherichia coli.*** Several different biological processes in *E. coli* likely to be under influence by ethanolic *M. oleifera* whole leaf extract treatment which leads to lysis of the bacterial cell. The pathways that are blocked in *E. coli* by the ethanolic *M. oleifera* whole leaf extract treatment include Ribosome production, and Translation. The pathways that are enhanced by the ethanolic *M. oleifera* whole leaf extract treatment in *E. coli* include Metabolism, DNA packaging, DNA fragmentation, Membrane Instability, Oxidative stress, and Cell lysis.

## Vita

Brandon Edward Smith was born in the fall of 1991 in Winston-Salem, North Carolina, to parents Debbie and Mickey Smith with one younger brother Justin Shane Smith. Brandon graduated from West Davidson High School in 2009 after receiving the Leadership Award and began his college career at Appalachian State University in August of that same year, with the hopes of pursuing a major in science. Brandon quickly realized he wanted to pursue a career in biology and rapidly specified his major as cellular/molecular biology pre-professional degree in the fall of his sophomore year. Brandon graduated cum laude from Appalachian State University in May 2013, with a Bachelor of Science degree in Biology, pre-professional, with a minor in chemistry and a minor in psychology.

Brandon had hopes of attending medical school after graduation but was reluctant to hold it off in the presence of wanting more exposure to research and the need for more shadowing hours within the health field. In the spring of 2014, Brandon began working towards his Master of Science degree in Cell and Molecular Biology, under the guidance and wisdom of Dr. Mowa. While working towards his Masters, Brandon attended scientific conferences in Boston and San Diego, attended national public health awareness week in DC, received basic EMT training, traveled to Zambia in Africa on a clinical shadowing independent study, and did medical volunteering in Peru. After graduation, Brandon will continue to increase his shadowing hours while acquiring a job as a medical scribe, lab assistant or medical assistant for several years while preparing for the MCAT and application process for Medical Schools. Brandon will then apply to medical schools until he is accepted.